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Diversity of the Ohio River Bacterial Communities Using Next-generation Sequencing Techniques

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DIVERSITY OF THE OHIO RIVER BACTERIAL COMMUNITIES USING NEXT-GENERATION SEQUENCING TECHNIQUES

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By

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And finally, but certainly not least, I dedicate my thesis to my advisor and committee members. Thank you Dr. Schultz, Dr. Somerville, and Dr. Trzyna for always pushing me to discover more and to expand my thoughts about microbiology past the traditional thinking. The years at Marshall University have been challenging yet very rewarding; I appreciate every piece of advice you three have given to me over the years.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

DIVERSITY OF THE OHIO RIVER BACTERIAL COMMUNITIES USING NEXT-GENERATION SEQUENCING TECHNIQUES.

Emily Michele Anneken

The ability to sequence bacterial genetic material directly from environmental samples has unlocked the field of metagenomics. Next-generation sequencing techniques, such as pyrosequencing, have been instrumental in providing knowledge about bacterial communities. This study focused on identifying the bacterial diversity of the Ohio River via pyrosequencing and compared the diversity of cultivable bacteria versus the entire river community. To ensure the maximum number of cultivable bacteria was obtained, cultures were grown on nonselective media and incubated at river temperature. Bacterial DNA was extracted, sequenced, and classified. The dominant phyla for the Ohio River included Cyanobacteria (38-66% of the total), Actinobacteria (18-33%), and Proteobacteria (14-25%). In contrast, Proteobacteria was the dominant phylum (80-93%) of the cultivable bacteria, with Actinobacteria and Cyanobacteria accounting for less than 1% or uncultivable. Based on the results from this study, pyrosequencing is a highly useful technique for identifying large bacterial communities accurately from environmental samples.

CHAPTER 1

LITERATURE REVIEW.

INTRODUCTION.

Freshwater habitats, such as rivers, streams, and lakes, are essential ecosystems as they are sources of drinking water, recreation, and fisheries. Rivers play a large role in oxidation, storage, and release of carbon as well as in the recycling of other nutrients and minerals for the surrounding ecosystems[6, 15]. The microbial world found in large river ecosystems has been often overlooked, but can offer important insights to the health of the environment[28]. Identification of the microbial community—which should include all organisms from bacteria and fungi to viruses and parasites present in freshwater ecosystems—is crucial when trying to understand an environment.

Bacteria likely comprise the majority of the planet's biodiversity and mediate many environmental processes[24, 25, 31, 39]. Bacteria can exhibit taxonomic diversity and community composition similar to plants and animals and are of great importance for medicine, agriculture, and ecology[25]. Although microbes occupy every niche across the world, their numbers and roles in communities are not well understood; in fact, they have an important role in ecology because they provide matter and energy for higher levels of the food chain. A bacterial community is a complicated structure that is more than just a gathering of individuals; each individual organism has a role that develops the framework to create a functional community.

Bacterial communities in freshwater habitats often are comprised of phyla lineages that are unique to aquatic ecosystems and cannot be found in terrestrial or

marine environments[15, 33, 63]. Such phyla include Actinobacteria, Betaproteobacteria, Bacteroidetes, Verrucomicrobia, Alphaproteobacteria, Cyanobacteria, and Gammaproteobacteria[15, 63]. These phyla can provide information about the water's composition, productivity, and nutrient cycling.

For this particular study, the goal was to sample the Ohio River to get a profile of the bacterial diversity residing in the river via sequencing techniques so that future research could be conducted on bio-assessment and water quality. The Ohio River spans seven states and is a valuable resource for humans in the form of drinking water, recreation and transportation, as well as an essential resource for a balanced biodiversity throughout its watershed. At the base of this ecosystem is the bacterial community, which if fully understood, would help in maintaining this delicate balance. The identification of the diversity of the bacterial community residing in the Ohio River will provide valuable knowledge and understanding of the natural system and biological flow of the river. In addition to being a pilot project, it is also a methodology study comparing new sequencing techniques against traditional laboratory procedures.

HISTORY OF METAGENOMICS:

To undertake a project on a freshwater system as large as the Ohio River, it is necessary to use techniques that can handle a large sample size. Metagenomics refers to the culture-independent technology that studies genomes from a mixed microbial community from terrestrial, medical, freshwater and marine environments[13, 36, 42, 48]. Before the required technology, biologists relied on the

perfect mixture of nutrient requirements and temperatures to culture and study microorganism diversity in a given environment. This approach was not only challenging but also laborious and often produced biased results. From this limitation, the field of metagenomics was formed when Pace *et al*. first used the idea of extracting DNA directly from environmental samples in 1985[41]. Pace *et al.* created a way to analyze microbial DNA by using vectors such as *Escherichia coli* to replicate and clone the DNA, create libraries, and to then reconstruct whole genomes or study particular genes. Since it does not rely heavily on culture-based techniques, this field of microbiology goes beyond traditional sequencing, to provide a more in-depth profile of community genomics.

Extracting microbial DNA for analysis from an environmental sample can be approached in two ways: function-based or sequenced-based screening[14, 46, 48] (Figure 1). Function-based metagenomics is used for screening DNA libraries for particular phenotypes, such as salt tolerance, enzymatic activity, or even antibiotic production from the cloned DNA[9, 48]. Questions about physiology and ecology from a complex community could be readily answered using sequenced-based analysis to reconstruct whole genomes[20]. Once genes are isolated and sequenced, reference gene databases such as National Center for Biotechnology Information (NCBI) or Basic Local Alignment Search Tool (BLAST) are used for further taxonomic identification. Often the 16S rRNA gene is used for microbial identification based on its universal presence in bacteria, species sequence conservation and evolutional variability.

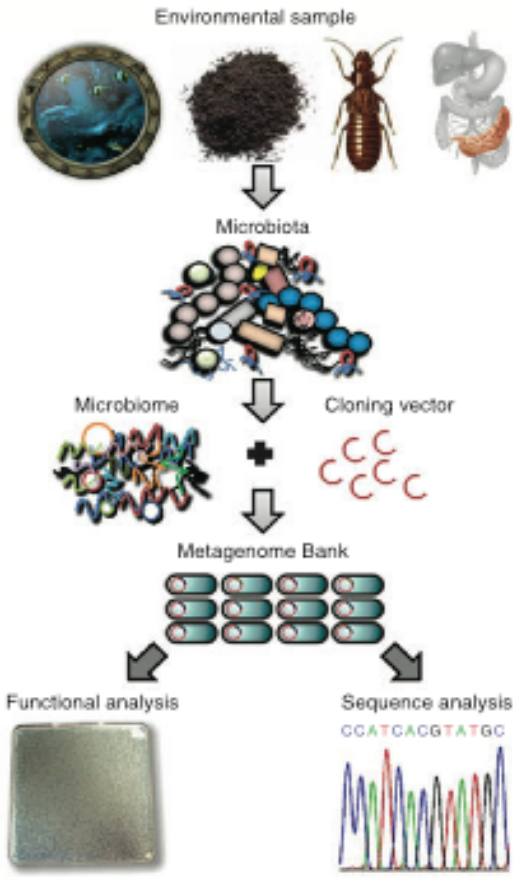

Figure 1. Metagenomic Approaches. Adapted from Sleator, R.D., *et al.* schematic overview of functional screening and sequence-based anaylsis[48].

The possibility of understanding distribution and functions within a community without experiencing the limitations of cultivation allowed for large-scale sequencing projects to discover the vast world of microorganisms, which was previously unattainable[20]. A particular study that demonstrated the advancement in microbiology made possible by the field of metagenomics was Craig Venter's study on the Sargasso Sea[55]. The purpose of Venter's study was to apply whole-genome shotgun sequencing using mixed microbial DNA obtained from a marine environment. Shotgun sequencing is a metagenomic technique in which the DNA is broken up into fragments and then re-assembled into complete stands. This study

identified approximately 1412 distinct small subunit rRNA genes and with a 99% similarity cutoff, 643 previously unidentified phylotypes (assemblages based on relatedness) were discovered[55]. The dominant taxa recovered were classified as Proteobacteria, Firmicutes, Cyanobacteria, Cytophaga Flavobacterium, and Bacteroidetes[55]. The amount of data obtained from this one study has taken years to process but has paved the way for other metagenomic studies to follow.

Although Venter's study has provided valuable insights into marine microbial diversity, the majority of knowledge regarding community diversity of freshwater systems comes from using denaturing gradient gel electrophoresis (DGGE) rather than shotgun sequencing[2, 40, 52]. DGGE works to identify microbial communities by creating genetic profiles of the dominant species within a population. The process requires an acrylamide gel having a low to high denaturing gradient, which will result in DNA strand separation[40]. This technique will classify the dominant organisms but is limited in identification of the whole community. These limitations have created the need for techniques that can be used effectively without the constraints and bias of traditional methods.

NEXT-GENERATION SEQUENCING: PYROSEQUENCING.

Next-generation sequencing refers to the high throughput, cost-effective sequencing techniques that can analyze large communities without the limitations of cultivation or traditional sequencing[42]. Such techniques can identify microbes quickly and does not rely on the amplification of the 16s rRNA to build phylogenetic

trees[37, 42]. Traditional methods, such as DGGE, terminal-restriction fragment length polymorphism (T-RFLP), or even Sanger sequencing of 16S rRNA cannot compare to the depth of identification that next-generation techniques can produce[47]. This is of particular interest because identifying entire communities can aid in understanding functional roles of microbes and classifying uncultivable bacteria within a given environment.

Pyrosequencing is a relatively new method of analyzing bacterial communities from environmental samples and can accurately identify all strains present in the sample. Marguiles *et al* was the first to introduce this novel sequencing technique in 2005 and showed its effectiveness in assembling bacterial genomes from short reads[38]. In addition, this technique did not require the cloning of bacteria, which before was a necessity for sequencing[38]. Pyrosequencing uses an enzymatic reaction to produce light to indicate when a nucleotide has been added to a growing strand of DNA[10, 38, 42]. The end result is a short sequenced strand that can then be used to determine the organism at the genus or species level from a mixed microbial environmental sample.

After Marguiles *et al,* Edwards *et al.* performed one of the very first studies conducted using pyrosequencing on the Soudan Mine groundwater. The water sampled from this mine is heavily rich in iron and the data showed the dominant phylum being Actinobacteria followed by Proteobacteria[10]. This study compared pyrosequencing to traditional techniques and found pyrosequencing to be more reliable and less costly[10].

Since Edwards' *et al.* project on the Soudan Mine, pyrosequencing has been used within all disciplines from medical settings and food industries to terrestrial and aquatic settings[10, 15, 27, 35, 42, 52-53, 55]. In relation to this paper's topic focus, a study conducted on seven different sites among brackish and oceanic environments used pyrosequencing V6 hypervariable region to pinpoint the bacterial communities present at each site[53]. The results showed sequences from 36 different phyla, with the dominant phyla being Proteobacteria, followed by Bacteroidetes, Cyanobacteria, and Actinobacteria making up 80% of the total[53]. The dominant phyla from the Amazonian site (salinity of 0) were found to be Actinobacteria and Acidobacteria. The results from this study suggested that there is a great diversity of new taxa that has yet to be described[53]. By combining phylogenic techniques with pyrosequencing, researchers can identify microbial species using the short sequence fragments that pyrosequencing provides[37].

Although this technology has been used for different ecosystems, there has been almost no application of pyrosequencing to large river systems such as the Ohio River. Recently, a study conducted by Ghai *et al*. used pyrosequencing to identify the microbial community residing in the Amazon River. The samples came from an area high in sediment concentrations but with a neutral pH during a dry season[15]. The samples were collected from mid-stream where the depth was approximately 8m, and free from pollutants or human influence[15].

The pyrosequencing results revealed a bacterial diversity comprising mostly of Actinobacteria and Proteobacteria[15]. Of the Actinobacteria, further identification of the 16S rRNA sequences was conducted and found that 73% were from the acI

lineage and the rest were from the acIV lineage, which are commonly found in freshwater systems,[15]. Of the Proteobacteria, the species were identified as belonging to Betaproteobacteria, which are well known and widely distributed among habitats[15, 19]. A third phylum found was Crenarchaea, which were previously known to occupy harsh habitats but now are thought to play a crucial role in ammonia oxidation in freshwater systems[5, 15].

The microbial community of the Amazon suggests an ecosystem highly efficient for processing organic matter mainly by Proteobacteria and Acidobacteria[15]. The indication for this was revealed by the overrepresentation of the Tannase domain, which is found in proteins involved in the degradation of tannins, and the Bug domain, found in proteins that are tricarboxylic acid receptors[1, 15, 21]. These protein domains can be located in both Acidobacteria and Proteobacteria, especially Beatproteobacteria, and suggest their importance in carbon cycling[15].

The conclusions drawn from this study alone have contributed knowledge about freshwater metagenomes using pyrosequencing that has not previously been reported. From this study, it is now known that Actinobacteria are primary members of freshwater ecosystems, the microbial community of freshwater can be remarkably different from marine or terrestrial environments, and that large-scale pyrosequencing studies can be conducted accurately and efficiently[15].

CULTIVATION:

Cultivation of bacteria in the laboratory has been a necessity to establish pure cultures from an environmental sample. Although this method has been a standard

in the field of microbiology, it is limited to only allowing the cultivable bacteria from a community to be cultured. A perfect example of how metagenomic sequencing can surpass cultivation is with the Human Microbiome Project[42]. The results from this project revealed that 62% of the bacteria identified were previously unknown and 80% were thought to be uncultivable[42]. The natural flora of the human intestines has been misunderstood due to cultivation but has now been corrected thanks to metagenomics.

There is often a large discrepancy between true populations and what can be diluted and cultivated when dealing with aquatic environments[20]. Relying on just cultivable bacteria will almost certainly misrepresent the true population of a community, with the possibility of the dominant organisms left unidentified due to lack of nutritional requirements or conditions that cannot be mimicked in the laboratory[35, 42]. Pyrosequencing can compensate for culture bias and produce results of the whole bacterial community present in the Ohio River. This study used pyrosequencing to determine bacterial diversity and compared it to the diversity of cultivatable bacteria from the same location in the river to illustrate the complex nature of the bacterial community in a large river ecosystem. In addition to the complexity, this study aims to express the magnitude of the importance of using a technique, such as pyrosequencing, when attempting to characterize the bacterial community of a natural ecosystem.

CHAPTER 2

MATERIAL & METHODS.

COLLECTION OF OHIO RIVER BACTERIAL SAMPLES.

Sample sites along the Ohio River were selected based on accessibility and named according to the location in the river. A total of eleven samples were taken from seven different sites in the river. Additionally, two samples were taken from the Guyandotte River upstream from where it empties into the Ohio River. Each sample was designated a number and labeled with the site location (Figure 2). GPS coordinates and water quality data, including Depth (m), Temperature (C°), Conductivity (uS), pH, pH measured in milivolts (pHmV), Turbidity (ntu), Chlorophyl-a (mg/L), dissolved oxygen percentage (odo%) and Volts (v) were recorded (Table 1).

Sterilized screw-capped bottles were used for collection of water samples (Figure 3). Each site had one or two bottles assigned to it for comparison of bacterial diversity. Three sample bottles taken from site location CH from the river channel were used for comparison of cultivable bacterial community versus entire community. To collect the bacterial community at each given site, the bottles were placed underwater, uncapped and filled with water, and then recapped before removing from the water. This method ensured that the water samples and the community within were without contamination.

CULTIVATION: PLATING.

Nonselective R2A media was prepared with 0.375µl of fungicide and poured aseptically in sterile Petri dishes. Petri dishes were placed in stacks of four, totaling three separate stacks for the three samples chosen for cultivation. Plates were labeled with the site and sample number and dated on the back of each dish. Water samples were swirled before 0.25ml of the sample was pipetted onto the center of each dish. Care was taken to prevent contamination. Glass spreaders were placed in a beaker with a small amount of ethanol at bottom and then were passed briefly through a flame to sterilize. Dishes were placed on a spin table and the lid of each was lifted and held over the dish during inoculation. The glass spreaders were used in the right hand to move the water sample over the surface of the plate until the sample was completely absorbed into media for each plate. The plates were incubated at river temperature for at least 48 hours. After incubation, all plates were removed from the incubator and placed on the lab bench at room temperature (Figure 4).

CULTIVATION: ISOLATION.

Plates were divided into four quadrants and each morphologically distinct colony within each quadrant was assigned a number according to the site, plate, quadrant, and colony. For example, the first colony collected from site three, plate one, quadrant one, first colony was given the identification number of 3:1:1:1, second picked was assigned 3:1:1:2 and so forth for the other colonies. Each morphologically distinct colony was then transferred to a new plate and incubated.

The plates were divided into eight triangular sections and the colony identification number was recorded on the back of the plate under the colony streak (Figure 5).

From these transferred colonies, a small portion was used to establish a DNA Library for future studies. A 100µl sample of R2A broth was placed into each well on a 96 well plate along with the bacteria and was incubated for 16 hours to make the DNA Library. After growth, 100µl of Glycerol solution was added to each well and lid was parafilmed, labeled, and frozen.

The isolated colonies that successfully grew were transferred to R2A media slants for long-term storage. From these colonies, the top thirteen morphologically different strains were further streaked for isolation onto R2A plates with fungacide. After several streaks for isolation, eight strains from the original thirteen strains could not be separated further. An additional DNA library was made using the previously mentioned method to preserve the DNA for future studies. The top eight different strains were subjected to Gram staining to determine cell morphology under oil immersion using a Zeiss compound microscope. Cell shape and structure for each strain were recorded.

DNA EXTRACTION: WATER SAMPLES.

A vacuum adapter and funnel were sterilized prior to use. Vacuum apparatus was set up and sterile forceps were used to place a 0.22µm Whatman Nuclear Track-Etch membrane filter onto the funnel. Water samples were decanted to 150ml and filtered. It was determined previously that the membrane filters could typically only support a total of 150ml of river water before becoming clogged.

After filtration, Mo Bio Powerwater DNA kit protocol was followed. Sterile forceps were used to remove the filter, which was then rolled so that the top was facing inward. Filter was placed in 5ml sterile bead collection tube supplied from Mo Bio Powerwater DNA kit and vortex horizontally with 1ml lysing reagent. Supernatant was removed and placed in a new collection tube and centrifuged for one minute. Supernatant was removed, transferred to a new tube with 200µl of a second reagent, placed in the fridge for five minutes and centrifuged for one minute. Supernatant was removed, transferred to a new tube with 650µl of a high salt concentration solution and vortex. Removed 650µl of supernatant and salt solution mixture, which was placed in a new tube with a spin filter membrane, centrifuged for one minute and discarded flow through. Repeated until all solution was centrifuged. Spin filter membrane was placed in a new tube, 650µl of alcohol-based wash was added, and centrifuged for one minute. Flow through was discarded and 650µl of a second wash was added then centrifuged for one minute. Flow through was discarded and membrane was centrifuged again for two minutes. Spin filter membrane was transferred to new collection tube, added 100µl of sterile elution buffer, and centrifuged for one minute. Membrane was discarded and extracted DNA was stored. After confirmation by gel electrophoresis (Figure 6-8), extracted DNA from each sample was stored in the freezer for further analysis.

DNA EXTRACTION: CULTIVABLE.

After observation of growth on each plate, colonies were removed from the surface of the media by a sterile cell scraper and placed in sterile flask filled with

150ml of water. Colonies were mixed in the water and flask was labeled with sample number located on back of the plates. Each flask containing the 150ml of water plus the cultivable colonies were filtered following the same procedure previously described. Mo Bio Powerwater DNA kit protocol was again followed and extracted DNA was frozen.

PYROSEQUENCING.

Isolated and purified DNA from each sampling site was shipped to Research and Testing Laboratories in Lubbock, Texas to undergo Pyrosequencing. The laboratory used the Roche 454 FLX Genome Sequencer system to perform the sequencing and identification of the mix DNA samples. Basic protocol for Pyrosequencing starts with hybridization of single stranded DNA to beads mixed with necessary enzymes (Figure 9). Nucleotides are added to the sample and incorporated onto the DNA strand if that nucleotide is the next in the sequence. ATP sulfurylase converts pyrophosphate to ATP, which reacts with enzyme Luciferase to produce light when a nucleotide has been added to the sequence. Each peak of light was detected and recorded. The intensity of the light corresponded to the number of nucleotides added to the sequence. Nucleotides not incorporated into the DNA strand are degraded. The process was then repeated with a different nucleotide and the entire enzymatic reaction continued. After undergoing the Pyrosequencing technique on 10,000 reads, all known DNA sequences were identified using NCBI database data were returned in Excel files for analysis.

SPECIES RICHNESS AND SIMPSON'S DIVERSITY INDEX.

Species richness is the number of different species in a given habitat. Species richness was calculated using the formula S=E+k(n-1/n) where S is species richness, E is the sum of the individuals for the sampling site, k is the number of unique/rare species at that site, and n is the number of species for the sample. The data for species richness, diversity, and similarity was sorted so that each species was represented for each site, even if the percentage value for a given species was zero. The sampling sites that had replicates, i.e. two or three sample bottles thus two or three values for one species, was averaged so that there would be only one value per species per site.

Data was sorted by site then the number of unique/rare species was found by checking each species percentage value for each site. For example, if the only percentage value for a given species was found for only one sampling site, that species was considered to be unique for that site; therefore this represented one (k) value. The data was analyzed until the correct number of unique/rare species (k) was found for each site. The number of species taxa per site was represented by (n) and was used in the equation (n-1/n). The number of individuals within a site was 100 and represented (E). Each (k), (E), and (n) value for a given site was placed in the equation S=E+k(n-1/n) and resulted in the species richness value for that site. This formula was repeated for each site. Data was recorded and compiled into a table.

Species diversity takes into account evenness as well as richness. For this study, Simpson's Diversity Index was calculated to find the probability that two organisms

sampled from a habitat will belong to different species. Simpson values range from 0 to 1, with values closer to 1 representing more evenness, meaning all species in the community are equal in numbers. The formula used was $D_s=1-\sum_i[n_i\ (n_i\ -1)]/[N*N-1]$, where n_i= the number of individuals of a species collected, N= the total number of organisms in a sample, and D_s= diversity.

Data was sorted by site and values for replicates were averaged to produce one value per species for each site. Averaged values represented (n), therefore were used to find n(n-1). The sum of n(n-1) was divided by 100 (N; total number of individuals for a given site) multiplied by 99 (N-1). The resulting value was the Simpson's Diversity value for the site. The formula was repeated for each site. Data was recorded and compiled into a table.

To determine if there is statistical difference between the Simpson's Diversity indexes, the variance was found and a t-test was conducted between each site. The formula used to find the variance was $s^2=4[p_i^3-(p_i^2)^2]N$, where p_i is the proportion of the number of organisms in a given species, essentially $p_i= n_i/N$.

Data was sorted by site and values for replicates were averaged to produce one value per species for each site. The averaged value for each species (n) was divided by 100 (N) and then raised to the power of three, which became the p_i^3 value. The averaged value for each species (n) was then divided by 100 (N) and then squared, which became the p_i^2 value. The sum of p_i^3 and p_i^2 values were found separately so there would be one p_i^3 and p_i^2 value for each site. The resulting values were then entered in the variance equation, $s^2=4[p_i^3-(p_i^2)^2]N$ and the variance was found. The equation was repeated until the variance for each site was found.

The Simpson's Diversity value and variance for each site were then used to calculate the t value. The equation used was $t=(Ds_1-Ds_2)/[sqrt(s_1^2+s_2^2)]$. The equation was repeated until each site was compared against each other. Data was recorded and compiled into a table.

Proportional Similarity was calculated to compare the similarity between the replicates at the sites and between the Ohio River sites versus the Guyandotte. The formula used was PS= ∑(lowest % value of a species between the communities). Data was sorted by site and values for replicates were averaged to produce one value per species for each site. The lowest percentage for each species between two or more sites was used, then the sum was found. The resulting value represented the proportional similarity. Data was recorded and compiled into a table.

Figure 2. Google Map Image of the seven sampling sites along the Ohio River and Guyandotte River. Located outside of Huntington West Virginia. OH=Ohio bank, CH= Channel, WV= West Virginia bank, GUY= Guyandotte River, CH GUY= Ohio River Channel at the mouth of the Guyandotte, OH GUY= Ohio bank across from mouth of the Guyandotte, and DS GUY= Ohio River downstream from Guyandotte.

Site	N	W	Depth (m)	Temp (C)	µs	pH	pHmV	ntu	mg/L	pcs (pc/nL)	odo %	v
OH	38 26m 20.8s	82 21m 24.6s	2.2	30.26	493	7.84	-45.7	10.3	3	3200	90.1	12.3
WV	30 26m 0.79s	82 21m 21.1s	2.7	29.95	495	7.76	-41.2	15.2	2.6	3700	82.6	12.3
CH	38 26m 17.65	82 21m 32.7s	6.9	30.12	491	7.78	-42.2	9.6	2.6	3000	84	12.4
GUY	38 25m 38.5s	82 23m 28.6s	2.8	25.58	223	7.62	-32.8	134.7	4.7	2500	81.1	12.3
CH GUY	38 26m 00.1s	82 23m 32.2s	6.4	30.1	494	7.8	-43.8	10.3	3.1	4000	86.1	12.3
OH GUY	38 26m 11.3s	82 23m 27.9s	4.1	29.9	504	7.73	-39.2	14	2.9	3450	81.2	12.3
DS GUY	38 26m 10.4s	82 24m 33.7s	6.4	29.84	499	7.74	-40	17	2.7	3500	82.8	12.4

Table 1. Water quality data taken at the day of sampling August 8th, 2010 on the Ohio River, outside of Huntington, West Virginia. The first two columns are the GPS coordinates, followed by Depth (m), Temperature (C°), Conductivity (uS), pH, pH measured in milivolts (pHmV), Turbidity (ntu), Chlorophyl-a (mg/L), dissolved oxygen percentage (odo%) and Volts (v).

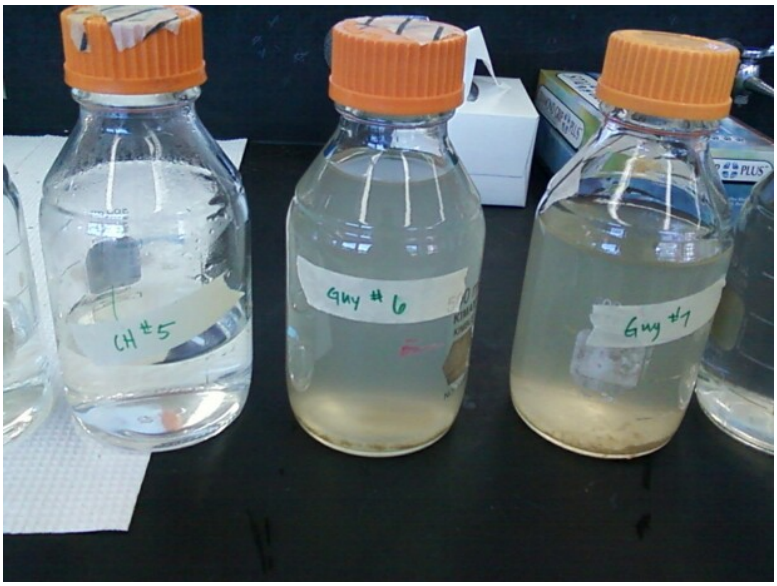

Figure 3. Screw-capped bottles used to collect water samples at each sampling site.

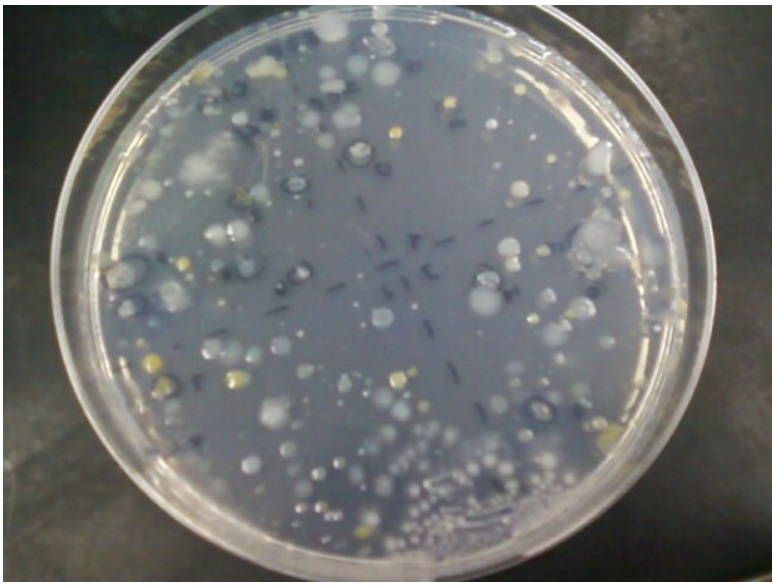

Figure 4. Cultivated plate after appropriate incubation.

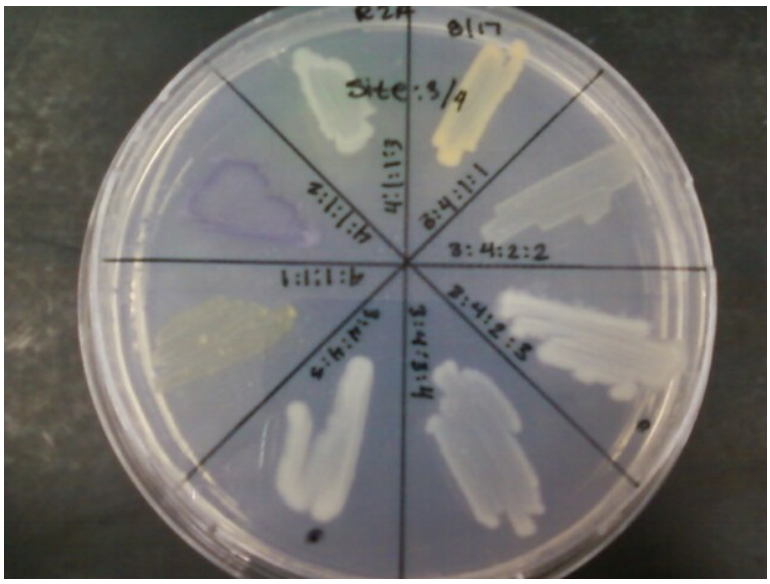

Figure 5. Petri dish with colony identification number.

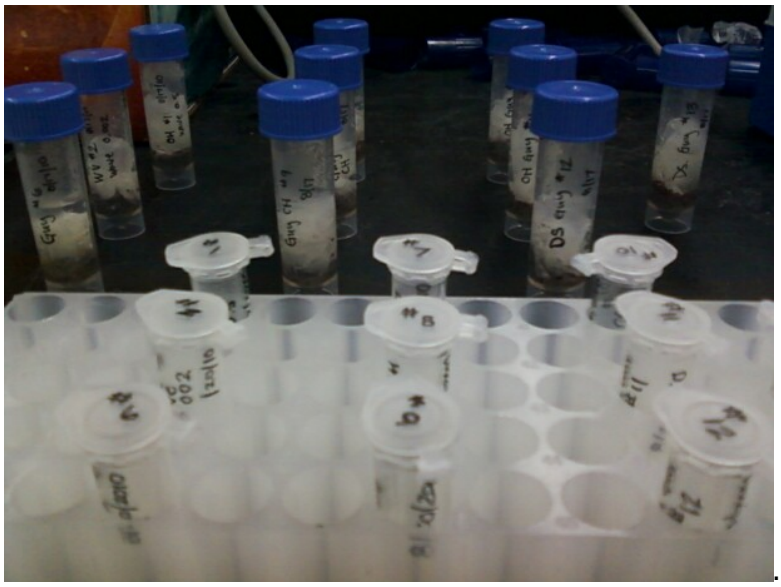

Figure 6. Filters used for DNA extraction.

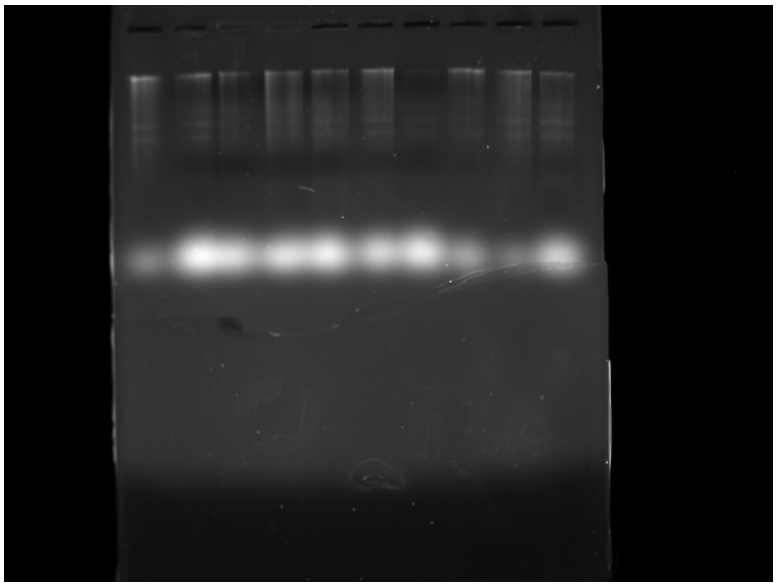

Figure 7. Gel electrophoresis of DNA extracted from sampling sites OH, WV, GUY, CH GUY, OH GUY, and DS GUY. The bright bands indicate that DNA was extracted successfully.

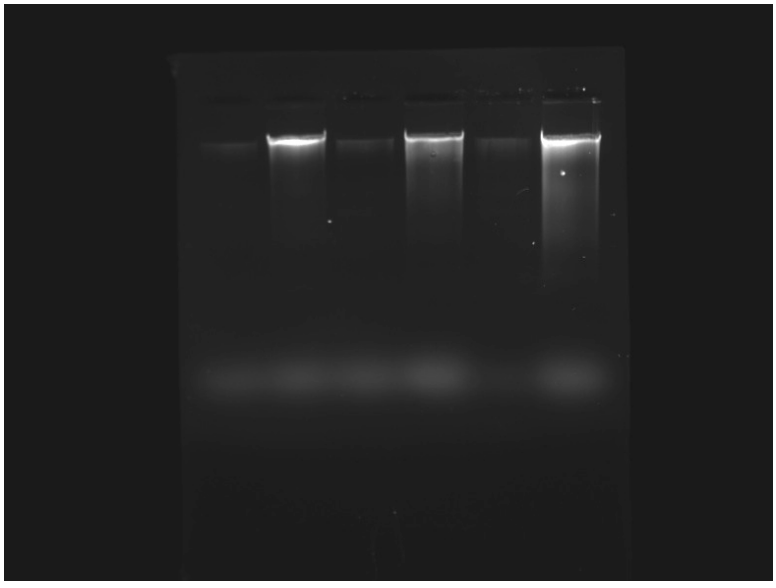

Figure 8. Gel Electrophoresis of DNA extracted from sampling site CH and of the cultivated community. The bright bands indicate that DNA was extracted successfully.

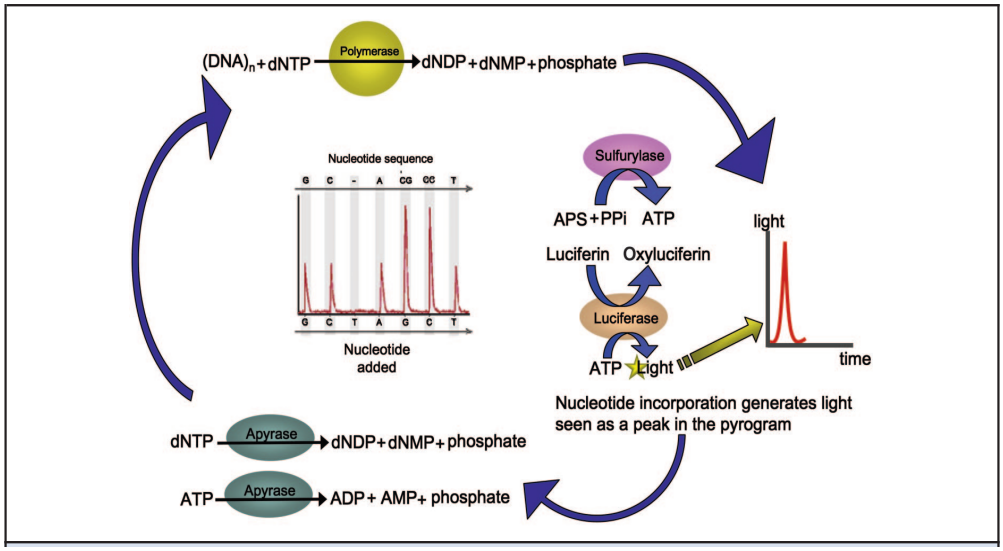

Figure 9. Pyrosequencing protocol. Adapted from Petrosino, J.F., *et al*. metagenomic pyrosequencing for microbial identification[42].

CHAPTER 3

RESULTS.

PYROSEQUENCING: COMMUNITY COMPOSITION AT PHYLA LEVEL.

Data obtained from pyrosequencing were organized in an Excel spreadsheet and analyzed. The phyla percentages for each sampling site were determined and complied into a graph for representation. The dominant phyla for the entire Ohio River bacterial community using pyrosequencing included Cyanobacteria (38.66-65.38% of the total), Actinobacteria (18.26-32.76%), and Proteobacteria (13.57-24.67%). For the Guyandotte, the dominant phyla were Proteobacteria (80.72% of the total), followed by Actinobacteria (12.45%), and Bacteroidetes (3.81%). Cyanobacteria were found to be 0.43% of the Guyandotte bacterial community. The complete phyla population percentages for all of the seven sampling sites can be viewed in Table 2 and Figure 10.

At each site, the dominant phyla vary slightly in percentage. The sample taken upstream on the Ohio bank—site labeled OH—phyla percentages included Cyanobacteria (65.38%), followed by Actinobacteria (18.66%), Proteobacteria (14.05%), Chloroflexi (0.59%), Bacteroidetes (0.55%), Bacteria (unknown phylum) (0.49%), Verrucomicrobia (0.46%), OP10 (0.15%), Gemmatimonadetes (0.14%) to Acidobacteria and WS3 (0.02%). See Figure 11.

For site WV, taken on the West Virginia bank opposite of the OH sampling site across the river, the phyla percentages range from Cyanobacteria (64.86%), followed by Actinobacteria (18.26%), Proteobacteria (14.66%), Bacteroidetes (0.80%), Chloroflexi (0.60%), Bacteria (unknown phylum) (0.52%),

Verrucomicrobia (0.50%), Gemmatimonadetes (0.16%), WS3 (0.07%), OP10 (0.06%), Firmicutes (0.03%) to Bacteria incertae sedis (0.02%). See Figure 12.

For site CH, taken from middle of the Ohio River, the phyla percentages range from Cyanobacteria (38.66%), followed by Actinobacteria (32.76%), Proteobacteria (24.67%), Bacteroidetes (1.37%), Verrucomicrobia (1.21%), Chloroflexi (0.90%), Bacteria (unknown phylum) (0.53%), Gemmatimonadetes (0.26%), OP10 (0.10%), Nitrospirae (0.03%) to WS3, Firmicutes, and Bacteria incertae sedis (0.02%). See Figure 13.

For site GUY, which is upstream from the mouth of the Guyandotte before it meets the Ohio River, the phyla percentages range from Proteobacteria (80.72%), followed by Actinobacteria (12.45%), Bacteroidetes (3.81%), OP10 (1.03%), Verrucomicrobia (.72%), Bacteria (unknown phylum) (0.48%), TM7 (0.23%), Chloroflexi (0.19%), Gemmatimonadetes (0.11%), Acidobacteria (0.08%), Firmicutes (0.06%), Nitrospirae (0.05%), OD1 (0.04%), WS3, Spirochaetes, and Bacteria incertae sedis (0.02%), to Candidatus Poribacteria (0.016%). See Figure 14.

For site CH GUY, samples were taken in the middle of the channel of the Ohio River just above where the Guyandotte enters the Ohio. The phyla percentages range from Cyanobacteria (60.45%), followed by Actinobacteria (21.18%), Proteobacteria (16.16%), Bacteroidetes (0.79%), Verrucomicrobia (0.49%), Chloroflexi (0.59%), Bacteria (unknown phylum) (0.51%), Gemmatimonadetes (0.17%), OP10 (0.07%), Bacteria incertae sedis (0.06%), WS3 and Firmicutes (0.04%), to Planctomycetes (0.004%). See Figure 15.

Site OH GUY was located on the Ohio side of the river across from the mouth of the Guyandotte as it entered the Ohio River. The phyla percentages range from Cyanobacteria (57.53%), followed by Actinobacteria (21.35%), Proteobacteria (18.58%), Bacteroidetes (0.80%), Chloroflexi (0.69%), Verrucomicrobia (0.63%), Bacteria (unknown phylum) (0.52%), Gemmatimonadetes (0.16%), OP10 (0.09%), Bacteria incertae sedis (0.05%), Firmicutes (0.04%), WS3 (0.03%), Acidobacteria, Spirochaetes and Nitrospirae (0.01%), to BRC1 (0.006%). See Figure 16.

For the final sampling site, DS GUY, which was located downstream after the Guyandotte entered and mixed with the Ohio River, the phyla percentages range from Cyanobacteria (64.39%), followed by Actinobacteria (20.12%), Proteobacteria (13.57%), Bacteroidetes (0.80%), Chloroflexi (0.49%), Bacteria (unknown phylum) (0.42%), Verrucomicrobia (0.29%), Gemmatimonadetes (0.14%), OP10 (0.07%), WS3 (0.06%), Bacteria incertae sedis and Firmicutes (0.02%), Spirochaetes (0.01%), to Cercozoa (0.004%). See Figure 17 and Figure 18 for site comparison to location.

PYROSEQUENCING: COMMUNITY COMPOSITION AT SPECIES LEVEL.

The species percentages for site OH, starting with the phylum Cyanobacteria, included dominant species *Synechococcus sp 0BB22S0* (35.72% of the total) followed by *Synechococcus** (See Table 3 for symbol key) at 9.66%, *Synechococcus sp CCMP839* (4.05%), *Cyanobium sp LB03* (4.05%), *Synechococcus sp 0BB26S03* (3.34%), *Synechococcus‡* (1.86%), Chroococcaceae† (1.04%), Chroococcales" (0.57%), and Cyanobacteria' (0.38%). See Table 3 and Figure 19.

The dominant Actinobacteria species for site OH was Actinomycetales" (6.32%), followed by Microbacteriaceae† (5.55%), Actinobacteria' (4.42%), Sanguibacteraceae† (0.57%), Streptomycetaceae† (0.39%), Micrococcaceae† (0.26%), and Dermabacteraceae† (0.25%). See Table 4 and Figure 20.

Of the Proteobacteria for site OH, the dominant species was Rickettsiaceae† (2.77%) followed by Rhizobiales" (1.96%), Alphaproteobacteria' (1.30%), Polyangiaceae† (0.86%), Burkholderiaceae† (0.53%), *Cupriavidus‡* (0.47%), *Methylovorus‡* (0.45%), *Curvibacter gracilis* (0.36%), *Polynucleobacter necessarius* (0.34%), *Curvibacter‡* (0.32%), Comamonadaceae† (0.33%), Burkholderiales" (0.18%), *Ramlibacter‡* (0.14%), *Polynucleobacter‡* and Rhodocyclaceae† at (0.08%), Oxalobacteraceae† (0.04%), Rhodobacteraceae†, *Paracoccus‡* and *Aeromonas punctata* at (0.02%), and *Malikia granosa* (0.01). See Table 5 and Figure 21.

For site WV, the dominant species, beginning with the phylum Cyanobacteria, range from *Synechococcus sp 0BB22S0* (35.43%) followed by *Synechococcus** (9.02%), *Synechococcus sp CCMP839* (4.31%), *Cyanobium sp LB03* (4.25%), *Synechococcus sp 0BB26S03* (3.38%), *Synechococcus‡* (2.24%), *Chroococcaceae†* (0.94%), *Chroococcales"* (0.64%), to *Cyanobacteria'* (0.51%). See Table 3 and Figure 19.

Of the Actinobacteria, the dominant species was Actinomycetales" (6.18%), followed by Microbacteriaceae† (5.71%), Actinobacteria' (4.10%), Sanguibacteraceae† (0.53%), Streptomycetaceae† (0.36%), Micrococcaceae† (0.31%), and Dermabacteraceae† (0.15%). See Table 4 and Figure 20.

Of the *Proteobacteria* for site WV, the dominant species was Rickettsiaceae† (3.31%) followed by Rhizobiales" (2.82%), Alphaproteobacteria' (1.62%), Polyangiaceae† (0.72%), Burkholderiaceae† (0.49%), *Cupriavidus‡* (0.40%), Comamonadaceae† (0.34%), *Methylovorus‡* (0.33%), *P. necessarius* (0.25%), *C. gracilis* (0.24%), *Curvibacter‡* (0.23%), *Ramlibacter‡* (0.13%), Burkholderiales" (0.10%), *Polynucleobacter‡* (0.07%), Rhodocyclaceae† (0.06%), Oxalobacteraceae† (0.05%), *M. granosa* (0.03), *Paracoccus‡* and *Hydrogenophaga‡* at (0.02%), and Rhodobacteraceae† (0.01%). See Table 5 and Figure 21.

For site CH, the dominant species, beginning with the phylum *Cyanobacteria,* range from *Synechococcus sp 0BB22S0* (23.28%) followed by *Synechococcus** (4.05%), *Cyanobium sp LB03* (1.82%), *Synechococcus sp 0BB26S03* (1.55%), *Synechococcus sp CCMP839* (1.43%), *Synechococcus‡* (1.00%), Chroococcaceae† (0.61%), Cyanobacteria' (0.30%), to Chroococcales" (0.28%). See Table 3 and Figure 19.

Among the Actinobacteria, the dominant species range from Actinomycetales" (12.20%), followed by Microbacteriaceae† (9.08%), Actinobacteria' (7.89%), Sanguibacteraceae† (0.75%), Micrococcaceae† (0.70%), Streptomycetaceae† (0.51%), to Dermabacteraceae† (0.25%). See Table 4 and Figure 20.

The dominant Proteobacteria species for site CH was Rickettsiaceae† (5.72%) followed by Rhizobiales" (4.33%), Alphaproteobacteria' (2.73%), Polyangiaceae† (1.82%), *Methylovorus‡* (1.14%), *Cupriavidus‡* (0.87%), Burkholderiaceae† (0.56%), *P. necessarius* (0.53%), *C. gracilis* (0.52%), Comamonadaceae† (0.44%), *Curvibacter‡* (0.34%), *Ramlibacter‡* (0.27%), Burkholderiales" (0.18%), *Polynucleobacter‡*

(0.14%), Oxalobacteraceae† (0.08%), Rhodobacteraceae† (0.05%), Rhodocyclaceae† and *M. granosa* (0.03%), *Paracoccus‡, Xylophilus‡, Malikia‡* and *Hydrogenophaga‡* at (0.01%). See Table 5 and Figure 21.

The dominant Cyanobacteria for site GUY range from *Synechococcus sp 0BB22S0* (0.13%) followed by *Synechococcus sp 0BB26S03* (0.06%), Cyanobacteria' (0.03%), *Synechococcus**, *Synechococcus sp CCMP839, Cyanobium sp LB03* and Chroococcaceae† at (0.02%), to *Synechococcus‡* and Chroococcales" at (0.01%). See Table 3 and Figure 19.

The dominant Actinobacteria species for site GUY was Actinomycetales" (3.14%) followed by Streptomycetaceae† (2.62%), Actinobacteria' (1.67%), Microbacteriaceae† (1.44%), Dermabacteraceae† (0.90%), Sanguibacteraceae† (0.66%), and Micrococcaceae† (0.41%). See Table 4 and Figure 20.

The dominant Proteobacteria species for site GUY was *Curvibacter‡* (21.73%) followed by Comamonadaceae† (14.53%), *C. gracilis* (7.85%), *P. necessarius* (2.31%), *Malikia‡* (2.03%), Burkholderiaceae† (2.00%), Burkholderiales" (1.41%), *Ramlibacter‡* (1.38%), *Hydrogenophaga atypical* (1.26%)*, Hydrogenophaga‡* at (1.19%), *Polynucleobacter‡* (0.85%), Rhodocyclaceae† (0.83%), *Xylophilus‡* (0.74%), *M. granosa* (0.69%), *Cupriavidus‡* (0.65%), Oxalobacteraceae† (0.62%), Rhodobacteraceae*†* (0.61%), *Paracoccus‡* (0.56%)*, Aeromonas punctata* (0.55%)*, Methylovorus‡* (0.52%), Polyangiaceae† and Rhizobiales" (0.43%), and Alphaproteobacteria' (0.29%), and Rickettsiaceae† (0.16%). See Table 5 and Figure 21.

Among the phylum Cyanobacteria for site CH GUY, the dominant species range from *Synechococcus sp 0BB22S0* (34.06%) followed by *Synechococcus** (8.42%), *Synechococcus sp CCMP839* and *Cyanobium sp LB03* (3.43%), *Synechococcus sp 0BB26S03* (2.69%), *Synechococcus‡* (2.21%), Chroococcaceae† (0.95%), Cyanobacteria' (0.57%), to Chroococcales" (0.49%). See Table 3 and Figure 19.

Of the Actinobacteria, the dominant species was Actinomycetales" (7.71%), followed by Microbacteriaceae† (6.26%), Actinobacteria' (4.78%), Sanguibacteraceae† (0.54%), Micrococcaceae† (0.45%), Streptomycetaceae† (0.27%), and Dermabacteraceae† (0.19%). See Table 4 and Figure 20.

The dominant Proteobacteria species for site CH GUY was Rickettsiaceae† (3.35%) followed by Rhizobiales" (2.54%), Alphaproteobacteria' (1.86%), Polyangiaceae† (0.90%), *Pseudomonas trivialis* (0.61%), Burkholderiaceae† (0.55%), *Methylovorus‡* and Comamonadaceae† (0.44%), *Cupriavidus‡* (0.36%), *C. gracilis* (0.35%), *Curvibacter‡* (0.34%), Ramlibacter‡ (0.26%), *P. necessarius* (0.23%), Burkholderiales" (0.18%), *Polynucleobacter‡* (0.12%), Oxalobacteraceae† (0.06%), Rhodocyclaceae† *(0.04%),* Rhodobacteraceae† (0.03%), *Malikia‡, H. atypica* and *M. granosa* at (0.02%), *Paracoccus‡* and *Hydrogenophaga‡* at (0.01%). See Table 5 and Figure 21.

For site OH GUY, the dominant species, beginning with the phylum Cyanobacteria, range from *Synechococcus sp 0BB22S0* (31.22%) followed by *Synechococcus** (8.60%), *Cyanobium sp LB03* (4.29%), *Synechococcus sp CCMP839* (3.43%), *Synechococcus sp 0BB26S03* (2.57%), *Synechococcus‡* (1.95%),

Chroococcaceae† (0.84%), Chroococcales" (0.49%), to Cyanobacteria' (0.38%). See Table 3 and Figure 19.

Of the Actinobacteria, the dominant species was Actinomycetales" (7.93%), followed by Microbacteriaceae† (6.25%), Actinobacteria' (4.76%), Sanguibacteraceae† (0.52%), Micrococcaceae† (0.41%), Streptomycetaceae† (0.37%), and Dermabacteraceae† (0.20%). See Table 4 and Figure 20.

The Proteobacteria species for site OH GUY was Rickettsiaceae† (4.31%) followed by Rhizobiales" (2.92%), Alphaproteobacteria' (1.93%), Polyangiaceae† (0.98%), Burkholderiaceae† (0.64%), *Methylovorus‡* (0.47%), Comamonadaceae† (0.45%), *Cupriavidus‡* (0.37%), *C. gracilis* (0.34%), *Curvibacter‡* (0.33%), *P. necessarius* (0.30%), *Ramlibacter‡* (0.19%), Burkholderiales" (0.18%), *Polynucleobacter‡* (0.13%), Rhodocyclaceae† (0.08%), Rhodobacteraceae† (0.06%), Oxalobacteraceae† (0.05%), *Malikia‡, Hydrogenophaga‡,* and *Xylophilus‡*` at (0.02%), *H. atypica*, *Paracoccus‡* and *P. trivialis* at (0.01%). See Table 5 and Figure 21.

Among the phylum Cyanobacteria for site CH GUY, the dominant species range from *Synechococcus sp 0BB22S0* (30.26%) followed by *Synechococcus** (11.21%), *Cyanobium sp LB03* (5.46%), *Synechococcus sp CCMP839* (4.88%), *Synechococcus sp 0BB26S03* (3.50%), *Synechococcus‡* (2.79%), Chroococcaceae† (1.10%), Chroococcales" (0.59%), to Cyanobacteria' (0.44%). See Table 3 and Figure 19.

Of the Actinobacteria, the dominant species was Actinomycetales" (7.39%), followed by Microbacteriaceae† (5.96%), Actinobacteria' (4.26%),

Sanguibacteraceae† (0.47%), Micrococcaceae† (0.38%), Streptomycetaceae† (0.33%), and Dermabacteraceae† (0.20%). See Table 4 and Figure 20.

Of the Proteobacteria, the dominant species was Rickettsiaceae† (2.48%) followed by Rhizobiales" (1.92%), Alphaproteobacteria' (1.24%), Polyangiaceae† (0.65%), Burkholderiaceae† (0.54%), *Methylovorus‡* (0.32%), Comamonadaceae† (0.52%), *Cupriavidus‡* (0.36%), *C. gracilis* (0.39%), *Curvibacter‡* (0.50%), *P. necessarius* (0.27%), *Ramlibacter‡* (0.21%), Burkholderiales" (0.18%), *Polynucleobacter‡* (0.13%), Rhodocyclaceae† (0.08%), Rhodobacteraceae† (0.06%), Oxalobacteraceae† (0.05%), *Malikia‡, Hydrogenophaga‡,* and *Xylophilus‡*` at (0.02%), *H. atypica*, *Paracoccus‡* and *P. trivialis* at (0.01%). See Table 5 and Figure 21.

SPECIES RICHNESS AND SIMPSON'S DIVERSITY INDEX.

Species richness was calculated using the formula S=E+k(n-1/n) where S is species richness, E is the sum of the individuals for the sampling site, k is the number of unique/rare species at that site, and n is the number of species for the sample. For site OH the species richness was 102, where k=2 and E was 100 and n=187. For site WV the species richness was 102, where k=2 and E was 100 and n=194. For site CH the species richness was 111, where k=11 and E was 100 and n=183. For site GUY the species richness was 286, where k=186 and E was 100 and n=474. For site CH GUY the species richness was 112, where k=12 and E was 100 and n=226. For site OH GUY the species richness was 109, where k=9 and E was 100

and n=233. For site DS GUY the species richness was 110, where k=10 and E was 100 and n=230. See Table 6.

Species diversity takes into account evenness as well as richness. For this study, Simpson's Diversity Index was calculated to find the probability that two organisms sampled from a habitat will belong to different species. Simpson values range from 0 to 1, with values closer to 1 representing more evenness, meaning all species in the community are equal in numbers. The formula used was $D_s=1-\sum_i[n_i\ (n_i\ -1)]/[N*N-1]$, where n_i= the number of individuals of a species collected, N= the total number of organisms in a sample, and D_s= diversity. For site OH, the Simpson's Diversity index D_s= 0.86; for site WV D_s= 0.86; for site CH D_s= 0.92; for site GUY D_s= 0.93; for site CH GUY D_s= 0.87; for site OH GUY D_s= 0.88; for site DS GUY D_s= 0.88. See Table 7.

To determine if there is statistical difference between the Simpson's Diversity indices, the variance was found and a t-test was conducted between each site. The formula used to find the variance was $s^2=4[p_i^3-(p_i^2)^2]N$, where p_i is the proportion of the number of organisms in a given species, essentially $p_i= n_i/N$. The t-test values between each site are as follows: OH vs. WV= -0.1, OH vs. CH= -1.7, OH vs. GUY= -2.1, OH vs. CH GUY= -0.3, OH vs. OH GUY= -0.7, and OH vs. DS GUY= -0.7. For site WV vs. CH= -1.6, WV vs. GUY= -2.0, WV vs. CH GUY= -0.2, WV vs. OH GUY= -0.6, and WV vs. DS GUY= -0.7. For site CH vs. GUY= -0.5, CH vs. CH GUY= 1.4, CH vs. OH GUY= 1.0, and CH vs. DS GUY= 1.0. For Site GUY vs. CH GUY= 1.8, GUY vs. OH GUY=1.5, and GUY vs. DS GUY= 1.5. For Site CH GUY vs. OH GUY= 0.4, and CH GUY vs. DS GUY= 0.47. For the last comparison, OH GUY vs. DS GUY= -0.03. See Table 8.

Proportional Similarity was calculated to compare the similarity between the replicates at the sites and between the Ohio River sites versus the Guyandotte. The formula used was PS= ∑(lowest % value of a species between the communities). The proportional similarity (PS) between replicates for site CH was found to be 92.04%; for site GUY the PS= 82.97%; for site CH GUY the PS= 92.04%; for site OH GUY the PS= 92.22%; for site DS GUY the PS= 92.90%. Between the Ohio River sampling sites the similarity is 66.48% and between the Ohio River sites and the Guyandotte the similarity is 15.12%. See Table 9.

Proportional Similarity against each site was as follows: OH vs. WV= 94.92%; OH vs. CH= 71.23%; OH vs. GUY= 18.05%; OH vs. CH GUY= 92.17%; OH vs. OH GUY= 90.11%; WV vs. CH= 71.54%; WV vs. GUY= 17.10%; WV vs. CH GUY= 93.90%; WV vs. OH GUY= 91.41%; WV vs. DS GUY= 90.94%; CH vs. GUY= 21.04%; CH vs. CH GUY= 75.80%; CH vs. OH GUY= 77.94%; CH vs. DS GUY= 71.15%; GUY vs. CH GUY= 18.11%; GUY vs. OH GUY= 18.98%; GUY vs.. DS GUY= 18.39%; CH GUY vs. OH GUY= 94.22%; CH GUY vs. DS GUY= 89.42%; OH GUY vs. DS GUY= 90.05%. See Table 10.

PYROSEQUENCING: CULTIVABLE COMMUNITY STRUCTURE.

From water samples taken at site CH, a portion of the water was pipetted onto non-selective R2A plates and incubated. After growth was observed, the plates were filtered and DNA was extracted and sent off for pyrosequencing. The phyla population percentages for the cultivable bacterial community range from Proteobacteria (88.50%), Firmicutes (9.85%), Bacteroidetes (1.54%), Bacteria

(unknown phylum) (0.41%), Actinobacteria (0.11%), to Cyanobacteria, Verrucomicrobia and OP10 (0.003%). See Table 11.

The dominant cultivated species from phylum Proteobacteria range from *Aeromonas veronii* (21.20%), *Alishewanella‡* (10.14%), *Vogesella‡* (9.90%), *Aeromonas schubertii* (9.09%), Alteromonadaceae† (5.49%), *Aeromonas jandaei* (4.22%), *Enterobacter cloacae* (2.93%), Neisseriaceae† (2.55%), *Pseudomonas putida* (1.11%), *Aeromonas punctata* (1.08%), *Vogesella indigofera* (0.96%), Comamonadaceae† (0.84%), *Leclercia sp* (0.83%), Gammaproteobacteria' (0.78%), *Aeromonas hydrophila* (0.77%), *Mitsuaria chitosanitabida* (0.70%), Burkholderiaceae† (0.68%), *Samsonia erythrinae* (0.62%), Alteromonadales" *(*0.61%), Oxalobacteraceae† (0.59%), *Yokenella sp* (0.55%), *Pseudomonas‡* (0.52%), *Alishewanella fetalis* (0.48%), *Chitinibacter tainanensis* (0.45%), *Escherichia coli* (0.41%), Chromatiales" (0.32%), *Serratia marcescens* (0.28%), *Pseudomonas straminea* (0.28%), *Curvibacter gracilis* (0.11%), to Alphaproteobacteria' and *Cupriavidus‡* at (0.02%). See Table 12.

The dominant cultivated species from phylum Firmicutes range from *Bacillus thuringiensis* (5.50%), *Bacillus mycoides* (1.76%), *Bacillus weihenstephanensis* (0.93%), *Bacillus‡* (0.61%), to *Bacillus cereus* at (0.49%). The dominant species from the phylum Bacteroidetes/Chorobi group consists of Flavobacteriaceae† at (1.10%). See Table 12. All species with a percentage over (≥0.5%) were considered dominant for the cultivated. In comparison to the dominant Proteobacteria species from the sampling site CH, the cultivated percentages were found to be less than 1% or zero. See Table 13.

CULTIVATION: ISOLATION.

Of the strains cultivated, eight strains were morphologically different and chosen to be further isolated. Four rounds of streaking for isolation were conducted for each of the eight strains. A DNA library was made to preserve the strains for further research. The eight strains underwent gram staining to observe cell shape and for the possibility of symbiotic relationships within the strain. Staining was done after each round of isolation to observe changes. Exact hours between isolation and staining was not recorded but the time frame generally was within hours after the two-day incubation period.

For the first strain, 31311, the morphological characteristics of the colonies were purple with white irregular edges with a sticky appearance. The gram stain revealed three different gram-negative bacteria: the first had a rod cell shape in chains of two, second were cocci with no arrangement, and the third was cocci in chains. See Table 14 and Figure 22.

For strain 4129, the colonies were clear with smooth edges and four different gram-negative bacteria were observed: the first being cocci in chains, second being cocci in clusters, third cocci in a single arrangement, and the fourth being rods in single arrangement. See Table 14 and Figure 23.

For strain 4226, the colonies were white with wavy edges and a slime spreading across the surface of the plate. Four gram-negative cell shapes were observed, two of which were rods, one cocci and the fourth being spirilla shaped. One of the rod

shaped cells were in chains, the second being single in arrangement; the spirilla and cocci cell shapes were in a single cell arrangement. See Table 14 and Figure 24.

For strain 4238, morphologically the colonies were neon yellow in color with irregular edges, with three different gram-negative cell shapes observed. The first cell shape was cocci in a single cell arrangement, the second was rods in single arrangement and the third cell shape were thin rods in chains of two. See Table 14 and Figure 25.

For strain 4317, the colonies had a neon orange color with smooth edges. Three gram-negative cell shapes were observed under staining: the first was cocci in a single cell arrangement, the second were thin rods with no arrangement, and the third were squat rods in a single arrangement. See Table 14 and Figure 26.

For strain 4427, the color of the colonies was a translucent orange with a slimy coating. Under staining, three gram-negative cell shapes were observed, of which the first was rods in pairs of two, the second was rods in a single arrangement, and the third was cocci in a single arrangement. See Table 14 and Figure 27.

For strain 51418, the colonies were white in color with smooth edges and a sticky or waxy coating covering the colonies. The cell shapes that were observed revealed three cocci shaped cells of which two were gram-negative and one was gram-positive. The gram-positive cocci were in single cell arrangement, one of the gram-negative cocci was squat in shape in pairs of two, and the third cocci were in chains with a possible capsule. See Table 14 and Figure 28.

For the final strain of the eight, 5246, had neon green colored colonies with irregular edges and a slimy coating that dyed the medium. Three gram-negative cell

shapes were observed under gram straining: the first being cocci in chains with a possible capsule, the second was squat rods in clusters, and the third was thin rods with no arrangement. See Table 14 and Figure 29.

Phyla	OH (%)	WV (%)	CH (%)	GUY (%)	CH GUY (%)	OH GUY (%)	DS GUY (%)
Cyanobacteria	65.38	64.86	38.66	0.43	60.45	57.53	64.39
Actinobacteria	18.66	18.26	32.76	12.45	21.18	21.35	20.12
Proteobacteria	14.05	14.66	24.67	80.72	16.16	18.58	13.57
Bacteroidetes	0.55	0.80	1.37	3.81	0.76	0.80	0.80
Verrucomicrobia	0.46	0.50	1.21	0.72	0.49	0.63	0.29
Chloroflexi	0.59	0.60	0.90	0.19	0.59	0.69	0.49
Gemmatimonadetes	0.14	0.16	0.26	0.11	0.15	0.16	0.14
OP10	0.15	0.06	0.10	1.03	0.07	0.09	0.07
Firmicutes	0.00	0.03	0.02	0.06	0.04	0.04	0.02
WS3	0.02	0.07	0.02	0.02	0.04	0.03	0.06
Bacteria incertae sedis	0.00	0.02	0.02	0.02	0.06	0.05	0.02
Acidobacteria	0.02	0.00	0.00	0.08	0.00	0.01	0.00
Spirochaetes	0.00	0.00	0.00	0.02	0.00	0.01	0.01
Nitrospirae	0.00	0.00	0.03	0.05	0.00	0.01	0.00
TM7	0.00	0.00	0.00	0.23	0.00	0.00	0.00
OD1	0.00	0.00	0.00	0.04	0.00	0.00	0.00
Fusobacteria	0.00	0.00	0.00	0.005	0.00	0.00	0.00
Planctomycetes	0.00	0.00	0.00	0.00	0.004	0.00	0.00
Cercozoa	0.00	0.00	0.00	0.00	0.00	0.00	0.004
Candidatus Poribacteria	0.00	0.00	0.00	0.016	0.00	0.00	0.00
BRC1	0.00	0.00	0.00	0.00	0.00	0.006	0.00
Bacteria (unk phylum)	0.49	0.52	0.53	0.48	0.51	0.52	0.42

Table 2. Phyla population averaged percentages for each sampling site. A site with replicates, i.e. two or more samples resulting in two or more values for one site, was averaged to produce one value per site. The seven sampling sites along the Ohio River and Guyandotte River, located outside of Huntington West Virginia, are OH=Ohio bank, CH= Channel, WV= West Virginia bank, GUY= Guyandotte River, CH GUY= Ohio River Channel at the mouth of the Guyandotte, OH GUY= Ohio bank across from mouth of the Guyandotte, and DS GUY= Ohio River downstream from Guyandotte.

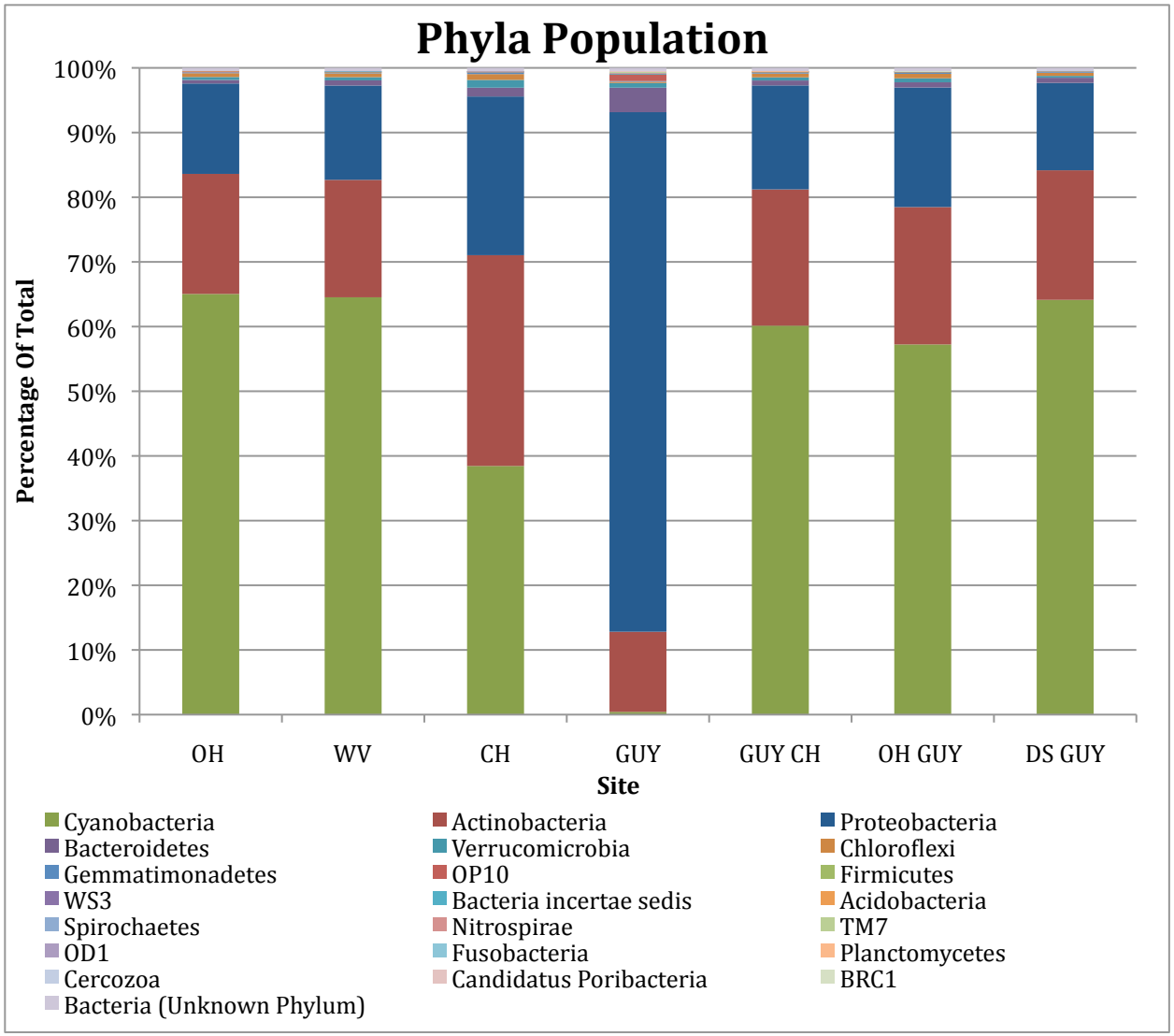

Figure 10. Phyla population percentages for each sampling site. Data from Table 2, Phyla Population.

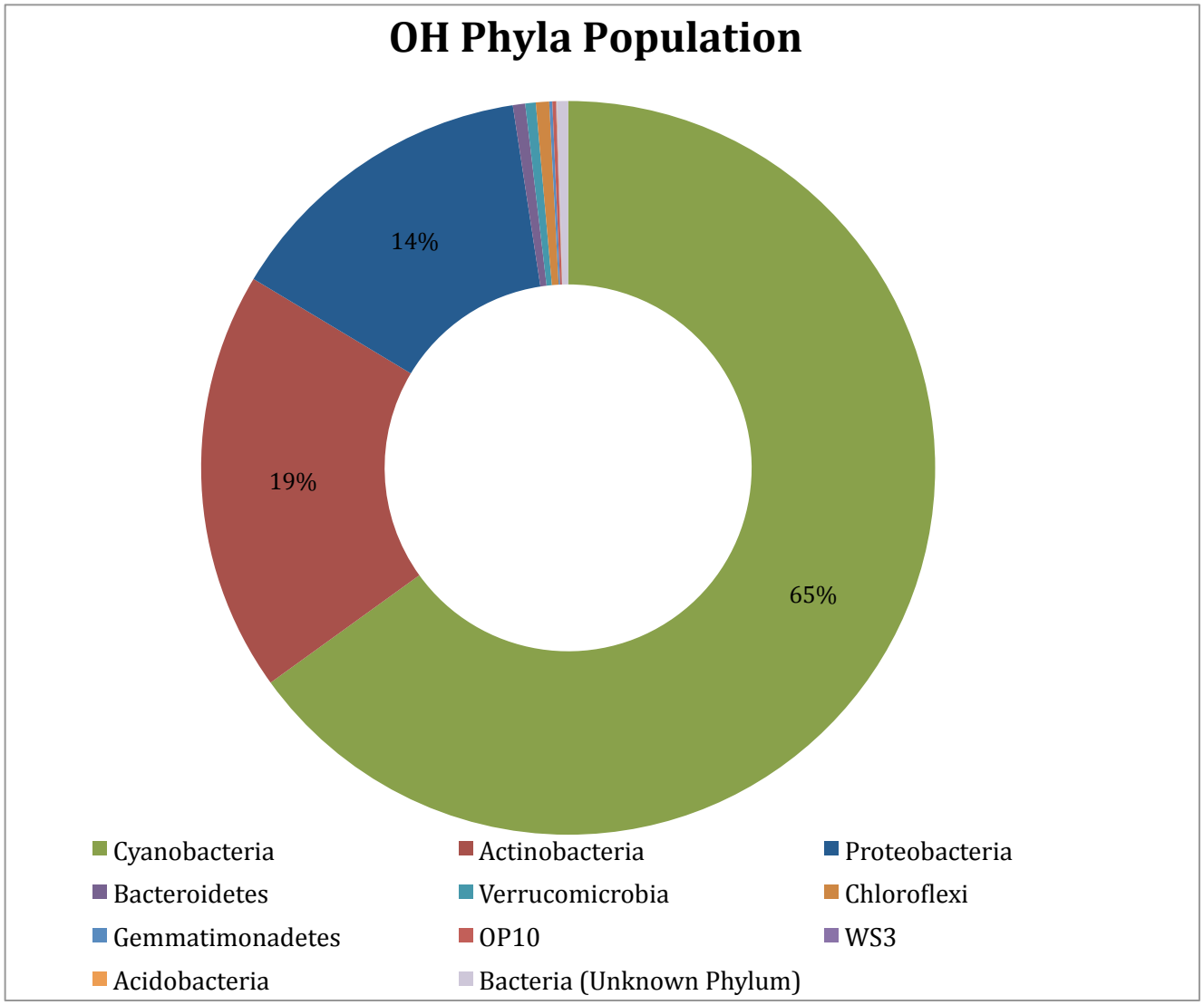

Figure 11. Sampling Site OH, taken from the Ohio bank along the Ohio River, phyla population percentages.

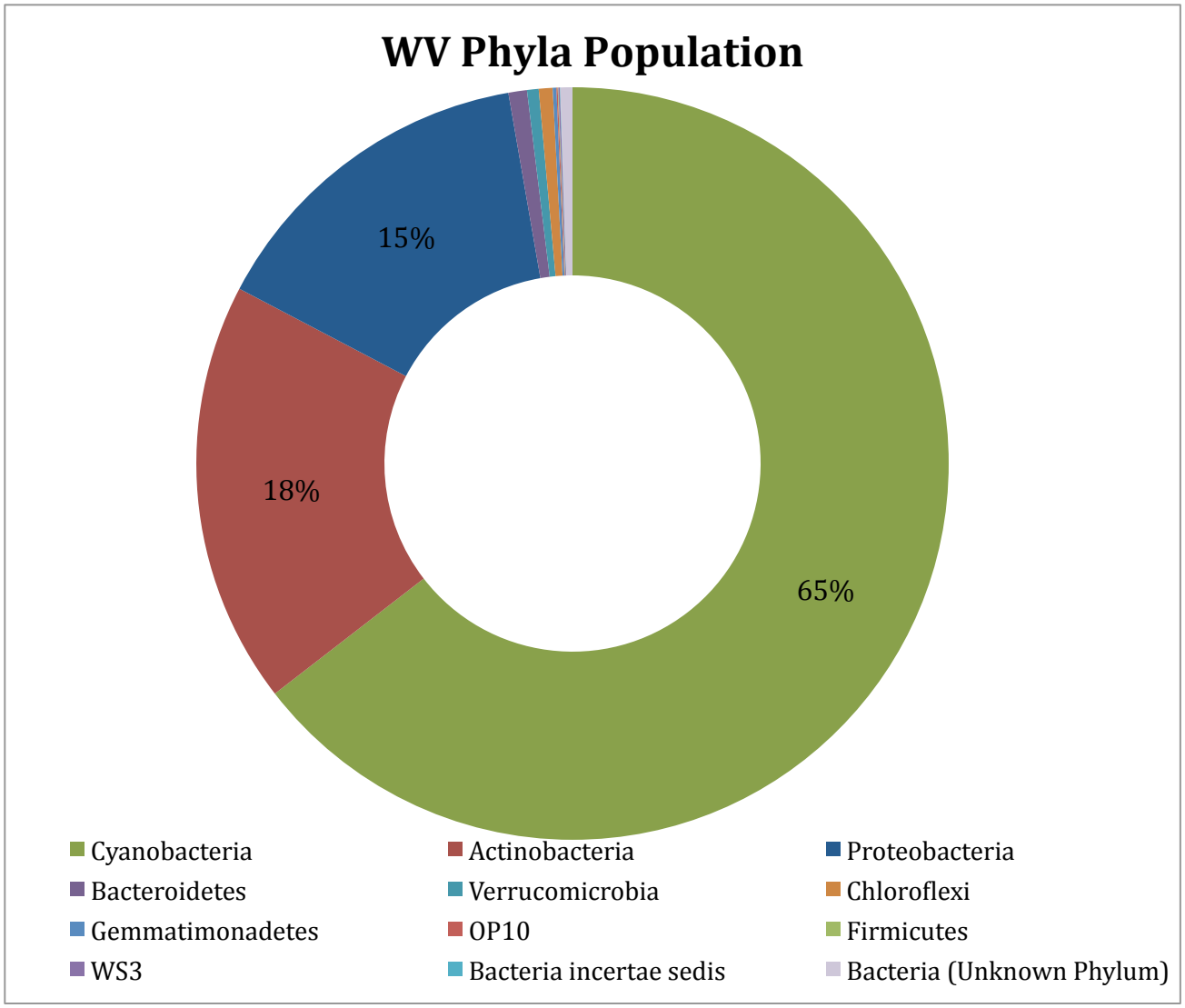

Figure 12. Sampling site WV, taken from the West Virginia bank of the Ohio River, phyla population percentages.

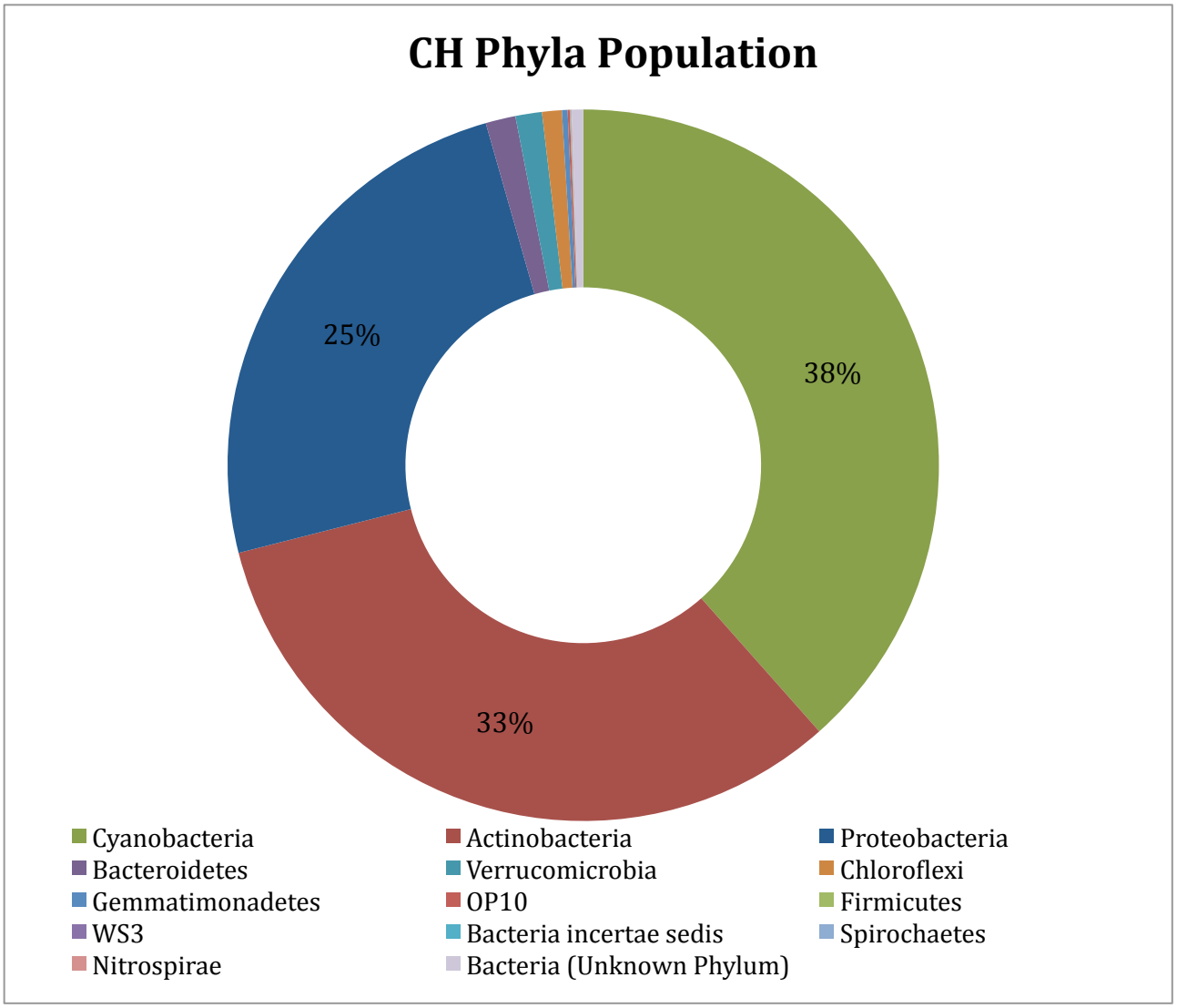

Figure 13. Sampling site CH, taken from the channel of the Ohio River, phyla population percentages.

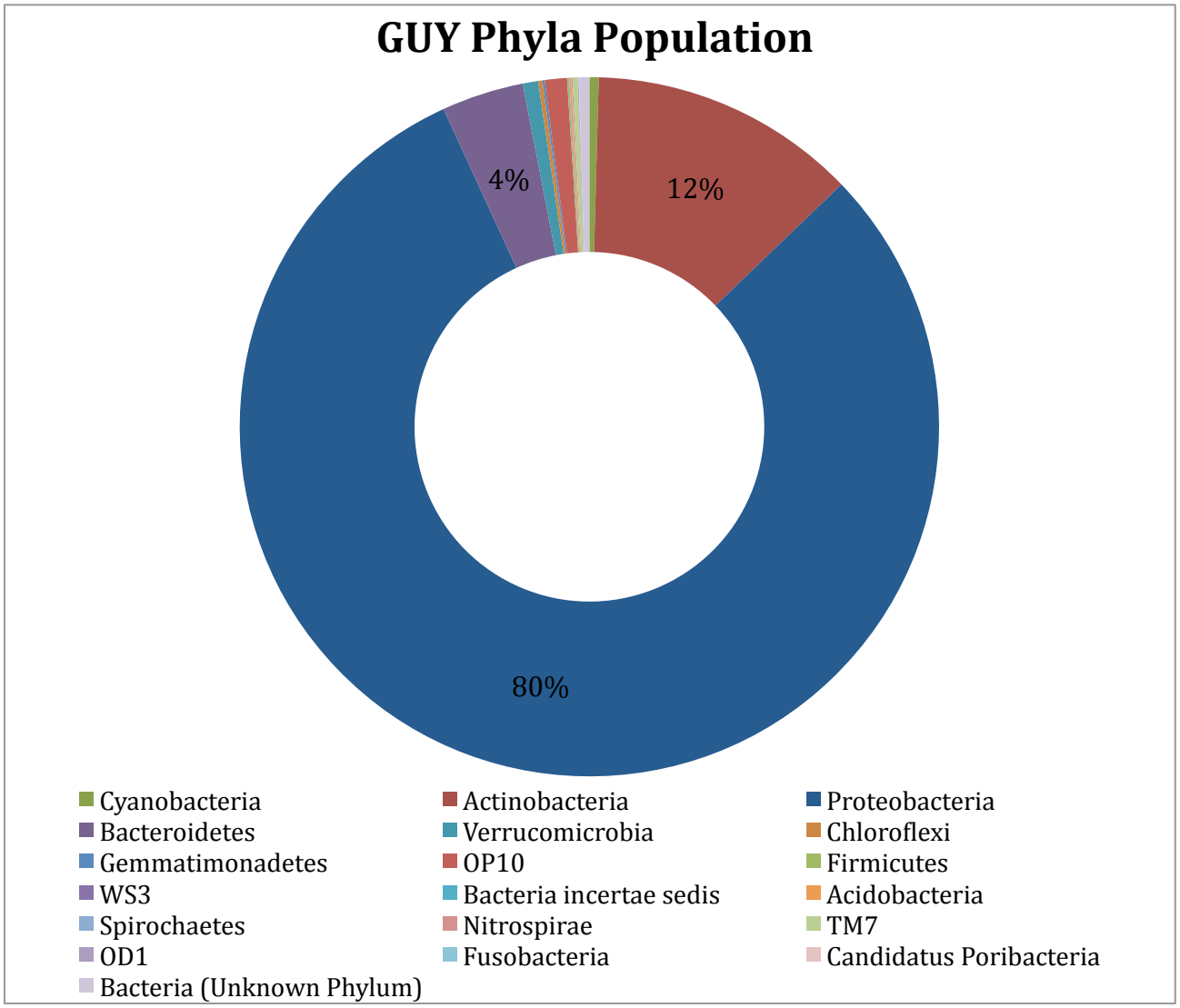

Figure 14. Sampling site GUY, taken from the Guyandotte River, phyla population percentages.

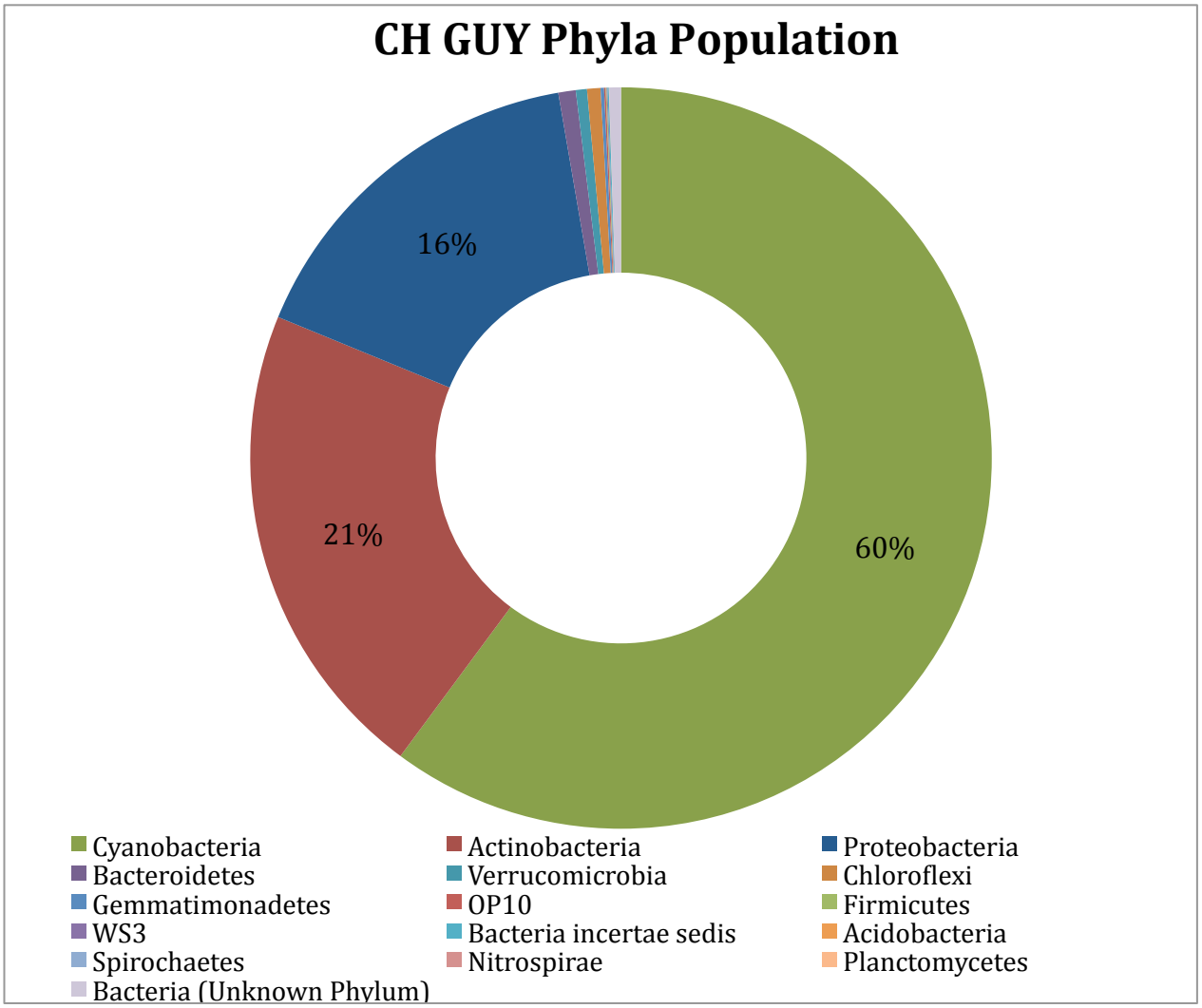

Figure 15. Sampling site CH GUY, taken from the channel in the Ohio River adjacent to the mouth of the Guyandotte, phyla population percentages.

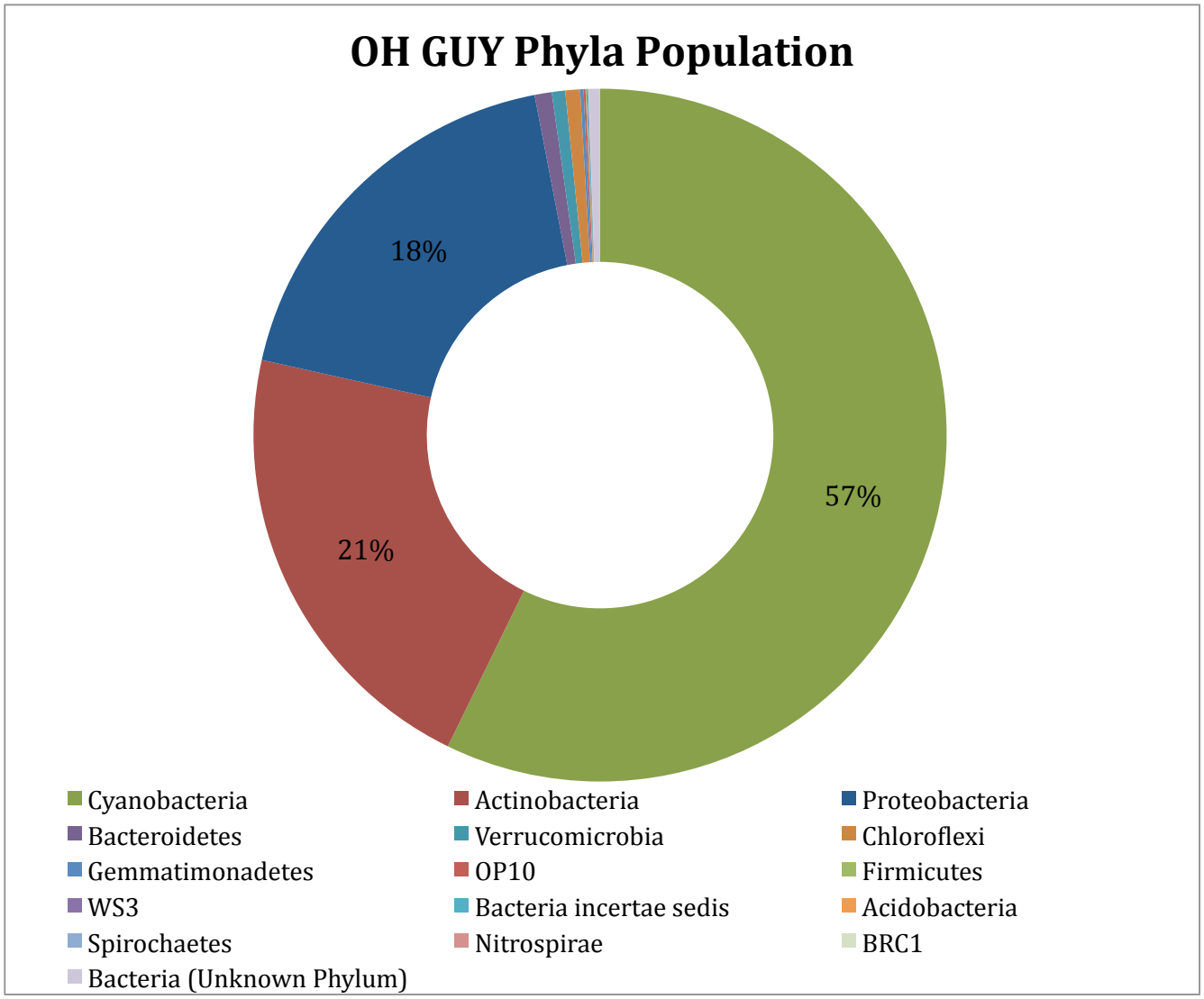

Figure 16. Sampling site OH GUY, taken from the Ohio bank across from the Guyandotte, phyla population percentages.

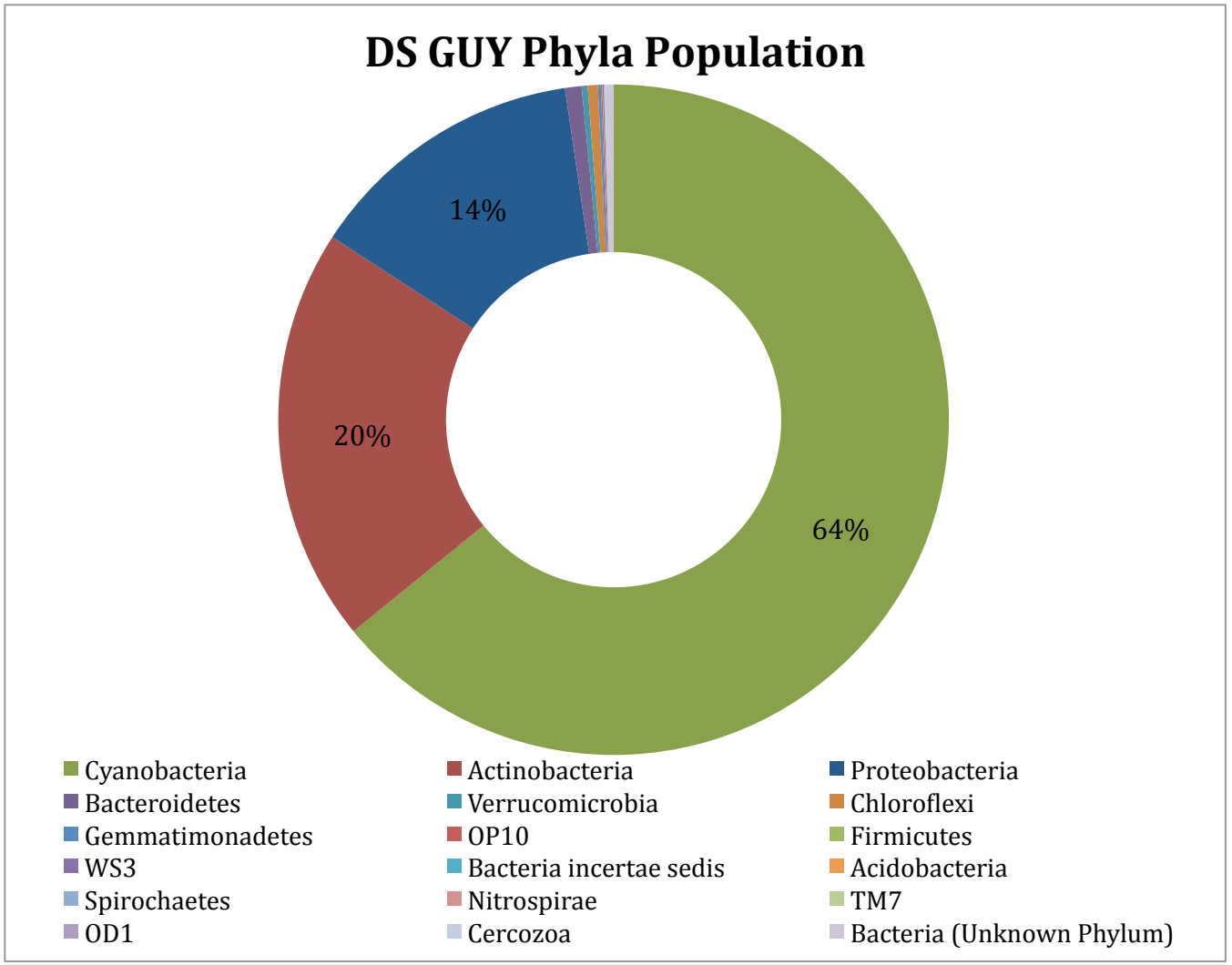

Figure 17. Sampling site DS GUY, taken downstream in the Ohio River from the Guyandotte, phyla population percentages.


OH #1
CH #3-5
WV #2
OH Phyla Population
14%
19%
65%
CH Phyla Population
25%
38%
33%
WV Phyla Population
15%
18%
65%
GUY Phyla Population
4%
12%
80%
Guyandotte
OH #10-11
CH GUY #8-9
OH GUY Phyla Population
18%
21%
57%
CH GUY Phyla Population
16%
21%
60%
DS GUY Phyla Population
14%
20%
64%
Image USDA Farm Service Agency

Figure 18. Phyla Population for each sampling site according to where each sample was taken from the Ohio River and Guyandotte. The seven sampling sites along the Ohio River and Guyandotte River, located outside of Huntington West Virginia, are OH=Ohio bank, CH= Channel, WV= West Virginia bank, GUY= Guyandotte River, CH GUY= Ohio River Channel at the mouth of the Guyandotte, OH GUY= Ohio bank across from mouth of the Guyandotte, and DS GUY= Ohio River downstream from Guyandotte. Refer to Figures 11-17 for phyla legend.

Dominant Cyanobacteria	OH (%)	WV (%)	CH (%)	GUY (%)	CH GUY (%)	OH GUY (%)	DS GUY (%)
Synechococcus sp 0BB22S0	35.72	35.43	23.28	0.13	34.06	31.22	30.26
*Synechococcus**	9.66	9.02	4.05	0.02	8.42	8.60	11.21
Synechococcus sp CCMP839	4.05	4.31	1.43	0.02	3.43	3.43	4.88
Cyanobium sp LB03	4.05	4.25	1.82	0.02	3.43	4.29	5.46
Synechococcus sp 0BB26S03	3.34	3.38	1.55	0.06	2.69	2.57	3.50
Synechococcus‡	1.86	2.24	1.00	0.01	2.21	1.95	2.79
Chroococcaceae†	1.04	0.94	0.61	0.02	0.95	0.84	1.10
Chroococcales"	0.57	0.64	0.28	0.01	0.49	0.49	0.59
Cyanobacteria'	0.38	0.51	0.30	0.03	0.57	0.38	0.44

Table 3. Dominant Cyanobacteria species population averaged percentages for each sampling site. Symbol Key: ‡unknown species, †unknown genus, "unknown family, ‘unknown order, ^unknown class, *uncultured.

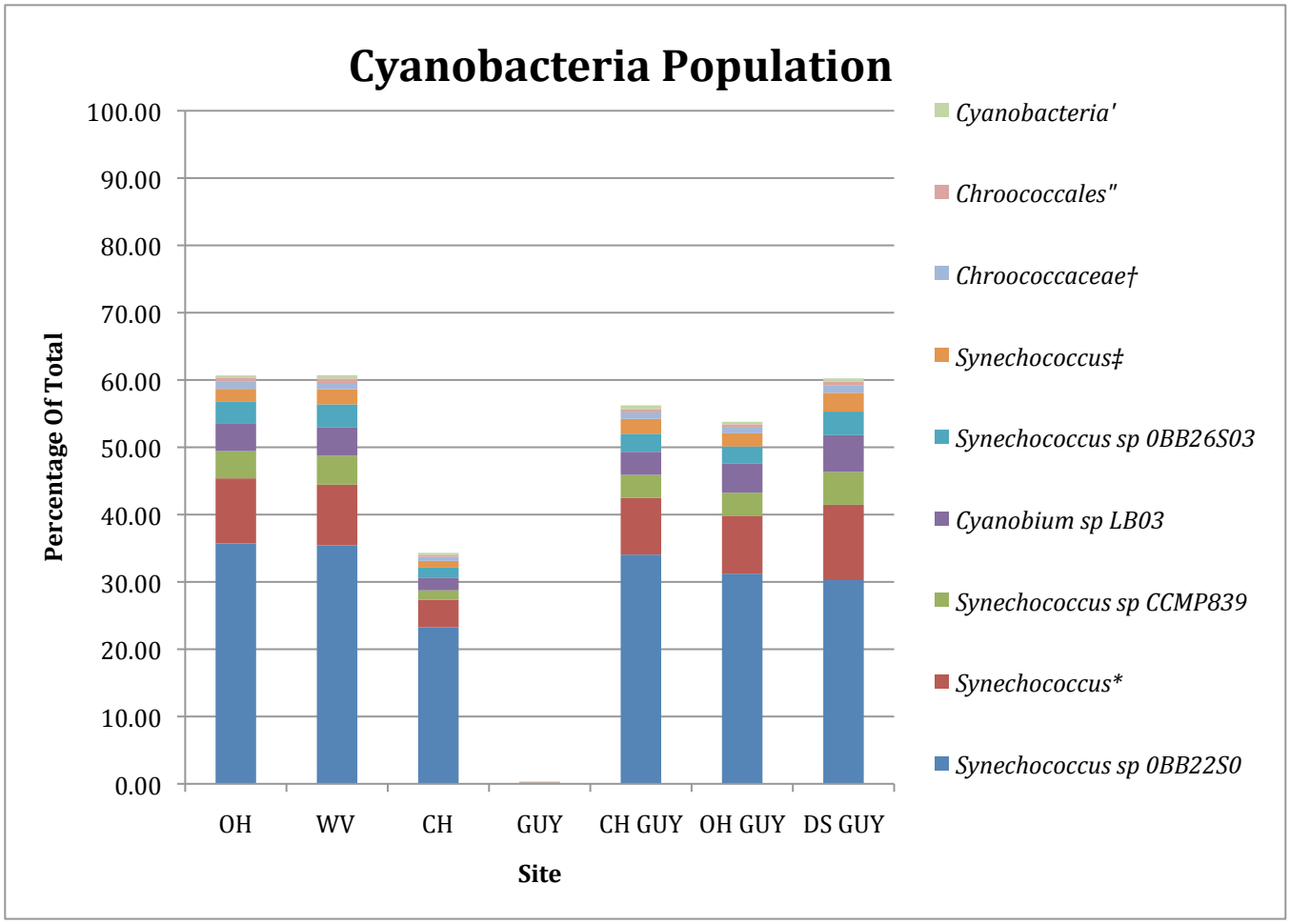

Figure 19. Dominant Cyanobacteria species population percentages for all sampling sites. Symbol Key: ‡unknown species, †unknown genus, "unknown family, ‘unknown order, ^unknown class, *uncultured.

Dominant Actinobacteria	OH (%)	WV (%)	CH (%)	GUY (%)	CH GUY (%)	OH GUY (%)	DS GUY (%)
Actinomycetales"	6.32	6.18	12.20	3.14	7.71	7.93	7.39
Microbacteriaceae†	5.55	5.71	9.08	1.44	6.26	6.25	5.96
Actinobacteria'	4.42	4.10	7.89	1.67	4.78	4.76	4.26
Sanguibacteraceae†	0.57	0.53	0.75	0.66	0.54	0.52	0.47
Micrococcaceae†	0.26	0.31	0.70	0.41	0.45	0.41	0.38
Streptomycetaceae†	0.39	0.36	0.51	2.62	0.27	0.37	0.33
Dermabacteraceae†	0.25	0.15	0.25	0.90	0.19	0.20	0.20

Table 4. Dominant Actinobacteria species population averaged percentages for each sampling site. Symbol Key: ‡unknown species, †unknown genus, "unknown family, ‘unknown order, ^unknown class, *uncultured.

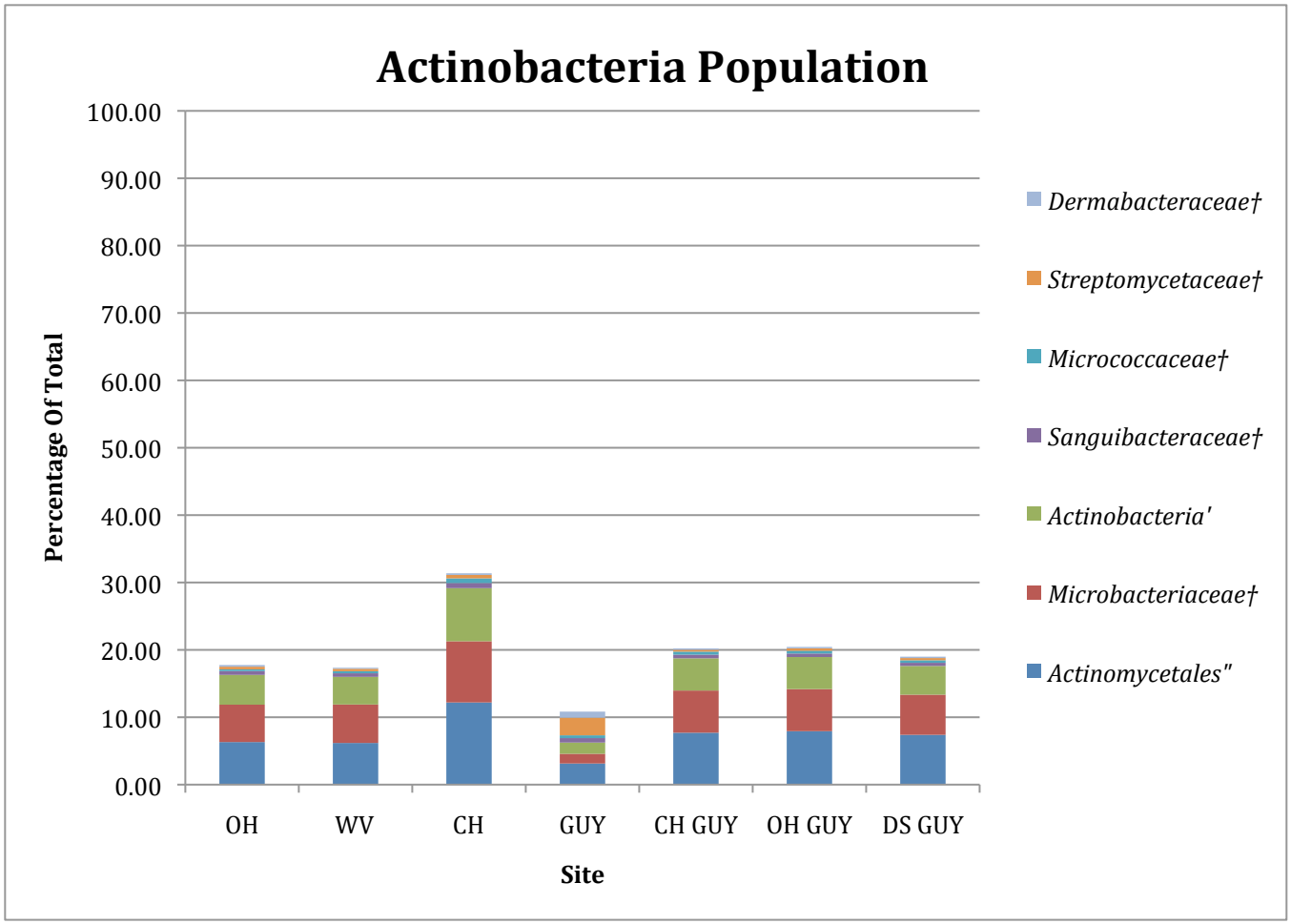

Figure 20. Dominant Actinobacteria species population percentages for each sampling site. Symbol Key: ‡unknown species, †unknown genus, "unknown family, ‘unknown order, ^unknown class, *uncultured.

Dominant Proteobacteria	OH (%)	WV (%)	CH (%)	GUY (%)	CH GUY (%)	OH GUY (%)	DS GUY (%)
Rickettsiaceae†	2.77	3.31	5.72	0.16	3.35	4.31	2.48
Rhizobiales"	1.96	2.82	4.33	0.43	2.54	2.92	1.92
Alphaproteobacteria'	1.30	1.62	2.73	0.29	1.86	1.93	1.24
Polyangiaceae†	0.86	0.72	1.82	0.43	0.90	0.98	0.65
Burkholderiaceae†	0.53	0.49	0.56	2.00	0.55	0.64	0.54
Methylovorus‡	0.45	0.33	1.14	0.52	0.44	0.47	0.32
Cupriavidus‡	0.47	0.40	0.87	0.65	0.36	0.37	0.36
Polynucleobacter necessarius	0.34	0.25	0.53	2.31	0.23	0.30	0.27
Curvibacter gracilis	0.36	0.24	0.52	7.85	0.35	0.34	0.39
Curvibacter‡	0.32	0.23	0.34	21.73	0.34	0.33	0.50
Comamonadaceae†	0.33	0.34	0.44	14.53	0.44	0.45	0.52
Malikia‡	0.00	0.00	0.01	2.03	0.02	0.02	0.02
Burkholderiales"	0.18	0.10	0.18	1.41	0.18	0.18	0.20
Ramlibacter‡	0.14	0.13	0.27	1.38	0.26	0.19	0.21
Hydrogenophaga atypica	0.00	0.00	0.00	1.26	0.02	0.01	0.02

Hydrogenophaga‡	0.00	0.02	0.01	1.19	0.01	0.02	0.03
Polynucleobacter‡	0.08	0.07	0.14	0.85	0.12	0.13	0.12
Rhodocyclaceae†	0.08	0.06	0.03	0.83	0.04	0.08	0.05
Xylophilus‡	0.00	0.00	0.01	0.74	0.00	0.02	0.01
Malikia granosa	0.01	0.03	0.03	0.69	0.02	0.00	0.02
Oxalobacteraceae†	0.04	0.05	0.08	0.62	0.06	0.05	0.04
Rhodobacteraceae†	0.02	0.01	0.05	0.61	0.03	0.06	0.06
Paracoccus‡	0.02	0.02	0.01	0.56	0.01	0.01	0.03
Aeromonas punctata	0.02	0.00	0.00	0.55	0.00	0.00	0.00
Pseudomonas trivialis	0.00	0.00	0.00	0.00	0.61	0.01	0.00

Table 5. Dominant Proteobacteria species population averaged percentages for each sampling site. Symbol Key: ‡unknown species, †unknown genus, "unknown family, ‘unknown order, ˆunknown class, *uncultured.

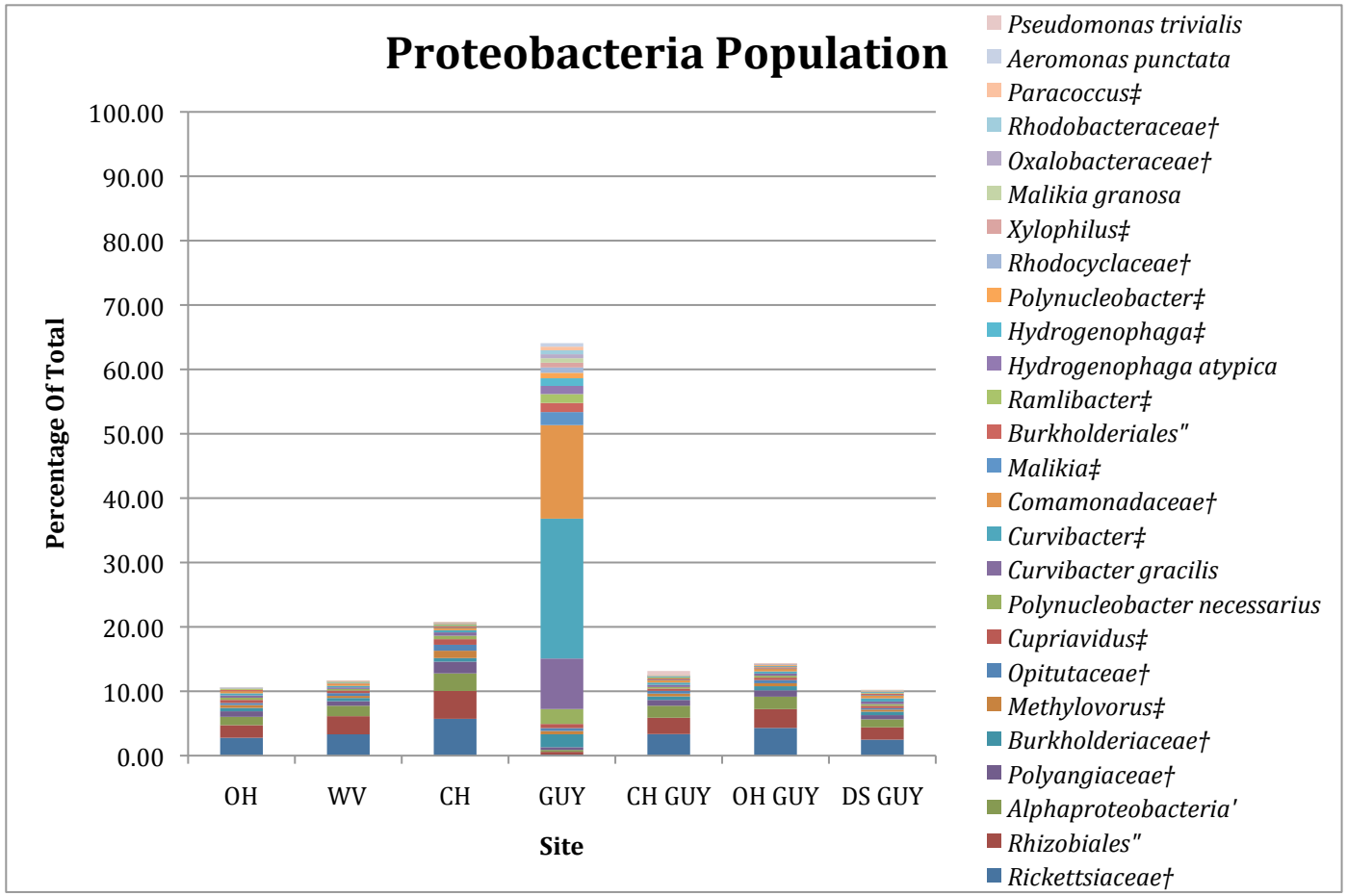

Figure 21. Dominant Proteobacteria species population percentages for all sampling sites. Symbol Key: ‡unknown species, †unknown genus, "unknown family, ‘unknown order, ˆunknown class, *uncultured.

Site	E	n	Unique/Rare (k)	Species Richness (S)
OH	100	187	2	102
WV	100	194	2	102
CH	100	239	11	111
GUY	100	474	186	286
CH GUY	100	226	12	112
OH GUY	100	233	9	109
DS GUY	100	230	10	110

Table 6. Species Richness for each sampling Site. E represents the sum of the individuals, n is the number of species for the sample, k is the number of unique/rare species at that site, and S is species richness.

Site	N	∑ n(n-1)	Simpson's Diversity (D_s)
OH	100	1428	0.86
WV	100	1406	0.86
CH	100	838	0.92
GUY	100	706	0.93
CH GUY	100	1317	0.87
OH GUY	100	1153	0.88
DS GUY	100	1141	0.88

Table 7. Simpson's Diversity Index, values ranging on a scale 0 to 1 with 1 representing more evenness. N is the total number of organisms in a sample and D_s is the diversity index

Site	OH	WV	CH	GUY	CH GUY	OH GUY	DS GUY
OH							
WV	-0.1						
CH	-1.7	-1.6					
GUY	**-2.1**	**-2.0**	-0.5				
CH GUY	-0.3	-0.2	1.4	1.8			
OH GUY	-0.7	-0.6	1.0	1.5	0.4		
DS GUY	-0.7	-0.7	1.0	1.5	0.47	-0.03	

Table 8. Diversity T-test matrix based on Simpson's diversity indexes for each site. Numbers in red indicate significant difference between the two compared sites.

Site	PS of Replicates (%)	PS of Ohio River Sites (%)	PS of Ohio River vs. Guyandotte (%)
OH	-	66.48	15.12
WV	-		
CH	92.04		
GUY	82.97		
CH GUY	92.04		
OH GUY	92.22		
DS GUY	92.90		

Table 9. Proportional Similarity (PS) between the replicates, i.e. two or more samples resulting in two or more values for the given site, and all sampling sites. Sites OH and WV did not have replicates, thus no PS value.

Site	OH (%)	WV (%)	CH (%)	GUY (%)	CH GUY (%)	OH GUY (%)	DS GUY (%)
OH							
WV	94.92						
CH	71.23	71.54					
GUY	18.05	17.10	21.04				
CH GUY	92.17	93.39	75.80	18.11			
OH GUY	90.11	91.41	77.94	18.98	94.22		
DS GUY	90.46	90.04	71.15	18.39	89.42	90.05	

Table 10. Proportional Similarity (PS) Matrix against each site.

Phyla	Cultivated (%)
Cyanobacteria	0.003
Actinobacteria	0.11
Proteobacteria	88.49
Bacteroidetes	1.54
Verrucomicrobia	0.003
Chloroflexi	0.00
Gemmatimonadetes	0.00
OP10	0.003
Firmicutes	9.85
WS3	0.00
Bacteria incertae sedis	0.00
Acidobacteria	0.00
Spirochaetes	0.00
Nitrospirae	0.00
TM7	0.00
OD1	0.00
Fusobacteria	0.00
Planctomycetes	0.00
Cercozoa	0.00
Candidatus Poribacteria	0.00
BRC1	0.00
Bacteria (unk phylum)	0.41

Table 11. Cultivated Phyla population averaged percentages. All phyla found in the Ohio River sampling sites were included with the percentages from the cultivated community.

Dominant Cultivated Species					
Proteobacteria	**%**	**Firmicutes**	**%**	**Bacteroidetes/Chorobi group**	**%**
Aeromonas veronii	21.20	*Bacillus thuringiensis*	5.50	Flavobacteriaceae†	1.10
Alishewanella‡	10.14	*Bacillus mycoides*	1.76		
Vogesella‡	9.90	*Bacillus weihenstephanensis*	0.93		
Aeromonas schubertii	9.09	*Bacillus‡*	0.61		
Alteromonadaceae†	5.49	*Bacillus cereus*	0.49		
Aeromonas jandaei	4.22				
Enterobacter cloacae	2.93				
Neisseriaceae†	2.55				
Pseudomonas putida	1.11				
Aeromonas punctata	1.08				
Vogesella indigofera	0.96				
Comamonadaceae†	0.84				

Leclercia sp	0.83
Gamma-proteobacteria'	0.78
Aeromonas hydrophila	0.77
Mitsuaria chitosanitabida	0.70
Burkholderiaceae†	0.68
Samsonia erythrinae	0.62
Alteromonadales"	0.61
Oxalobacteraceae†	0.59
Yokenella sp	0.55
Pseudomonas‡	0.52
Alishewanella fetalis	0.48
Chitinibacter tainanensis	0.45
Escherichia coli	0.41
Chromatiales"	0.32
Serratia marcescens	0.28
Pseudomonas straminea	0.28

Curvibacter gracilis	0.11				
Alpha-proteobacteria'	0.02				
Cupriavidus‡	0.02				

Table 12. Dominant species by phylum of the cultivated bacteria averaged percentages. Symbol Key: ‡unknown species, †unknown genus, "unknown family, ‘unknown order, ˆunknown class, *uncultured.

Proteobacteria Population Extracted CH vs. Cultivated CH		
Dominant (≥0.5%) CH Proteobacteria	**Extracted CH (%)**	**Cultivated CH(%)**
Rickettsiaceae†	5.72	0.00
Rhizobiales"	4.33	0.00
Alphaproteobacteria'	2.73	0.02
Polyangiaceae†	1.82	0.01
Methylovorus‡	1.14	0.00
Cupriavidus‡	0.87	0.02
Burkholderiaceae†	0.56	0.68
Polynucleobacter necessarius	0.53	0.01
Curvibacter gracilis	0.52	0.11
Dominant (≥0.5%) Cultivated Proteobacteria	**Cultivated CH (%)**	**Extracted CH (%)**
Aeromonas veronii	21.20	0.02
Alishewanella‡	10.14	0.09
Vogesella‡	9.90	0.06
Aeromonas schubertii	9.09	0.01
Alteromonadaceae†	5.49	0.03
Aeromonas jandaei	4.22	0.00
Enterobacter cloacae	2.93	0.00
Neisseriaceae†	2.55	0.01
Pseudomonas putida	1.11	0.03
Aeromonas punctata	1.08	0.00
Vogesella indigofera	0.96	0.00
Comamonadaceae†	0.84	0.44
Leclercia sp	0.83	0.00
Gammaproteobacteria'	0.78	0.05
Aeromonas hydrophila	0.77	0.00
Mitsuaria chitosanitabida	0.70	0.01
Burkholderiaceae†	0.68	0.56

Samsonia erythrinae	0.62	0.00
Alteromonadales"	0.61	0.01
Oxalobacteraceae†	0.59	0.08
Yokenella sp	0.55	0.00
Pseudomonas‡	0.52	0.01

Table 13. Proteobacteria population averaged percentages; Sampling site CH vs. cultivated community. Symbol Key: ‡unknown species, †unknown genus, "unknown family, ‘unknown order, ^unknown class, *uncultured.

Strain	Morphological Characteristics	Gram Stain	Cell Shape	Arrangement
31311	Purple with white. Sticky. Irregular Edges.	G-	Rods	Chained, pairs of two
		G-	Cocci	No arrangement
		G-	Cocci	Chains
4129	Clear with smooth edges.	G-	Cocci	Chains
		G-	Cocci	Clusters
		G-	Cocci	Single
		G-	Rods	Single
4226	White with wavy edges. Slimy.	G-	Rods	Chains
		G-	Spirilla	Single
		G-	Rods	Single
		G-	Cocci	Single
4238	Neon yellow with Irregular edges.	G-	Cocci	Single
		G-	Rods	Single
		G-	Rods	Thin, in chains of two
4317	Neon orange with smooth edges.	G-	Cocci	Single
		G-	Rods	Thin, no arrangement
		G-	Rods	Squat, Single
4427	Orange with slimy coating	G-	Rods	Pairs of two
		G-	Rods	Single
		G-	Cocci	Single
51418	White smooth edges. Sticky/waxy coating.	G+	Cocci	Single
		G-	Cocci	Squat, Pairs of two
		G-	Cocci	Chains with capsule
5246	Neon green with Irregular edges. Slimy.	G-	Cocci	Chains with capsule
		G-	Rods	Squat, clusters
		G-	Rods	Thin, no arrangement

Table 14. Cell Morphological features for the eight isolated strains from cultivation.

Figure 22. Strain 31311 colony morphology.

Figure 23. Strain 4129 colony morphology.

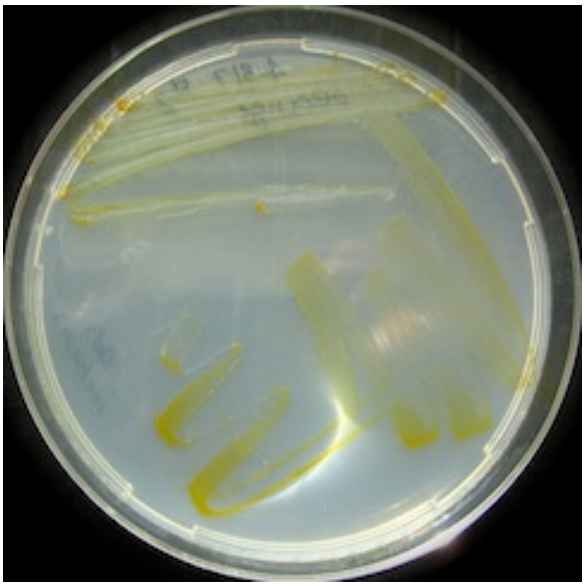

Figure 26. Strain 4317 colony morphology.

Figure 27. Strain 4427 colony morphology.

Figure 28. Strain 51418 colony morphology.

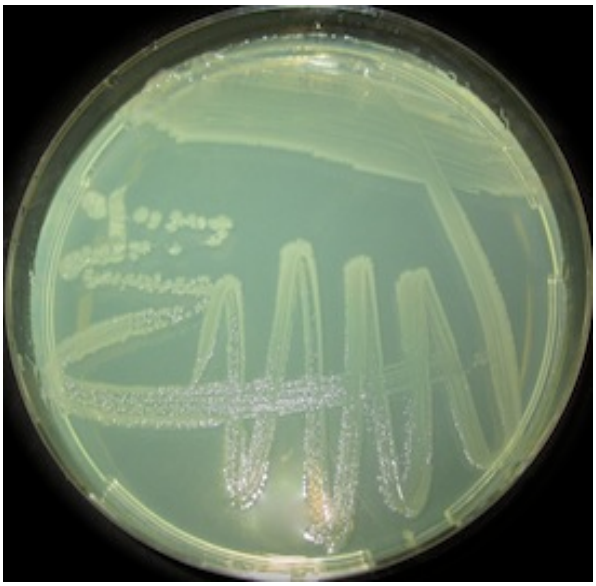

Figure 29. Strain 5246 colony morphology.

CHAPTER 4

DISCUSSION.

BACTERIAL COMMUNITY: OHIO RIVER.

Based on the results from pyrosequencing, the Ohio River is made up mainly of phyla from Cyanobacteria (38.66-65.38% of the total), Actinobacteria (18.26-32.76%), and Proteobacteria (13.57-24.67%). These three phyla play crucial roles in cycling nutrients and filtering the water for use by other organisms. It is important to stress that this thesis was a pilot study. It is currently unknown how the bacterial community of the Ohio River and the Guyandotte River are related to the overall health of the river.

CYANOBACTERIA: OHIO RIVER.

When taking a more in-depth look at this community, the dominant phylum was found to be Cyanobacteria, commonly known as blue-green algae. This phylum of bacteria is composed of photoautotrophic organisms that use water as an electron donor. Photopigments chlorophyll *a* and β-carotene that are essential for plant photosynthesis can be found within cyanobacteria and it is for this reason that they were originally named as algae; however, at the cellular and organismal level they resemble bacteria[51].

At the species level, the dominant Cyanobacteria found in the Ohio River are all part of the same order Chroococcales, which contains two families: Chroococcaceae and Entophysalidaceae. The species that were identified for the community belong to Chroococcaceae, which are coccoid or rod-shaped organisms that may form loose

colonies held together by a slime layer[51]. The dominant species for the Ohio River are *Synechococcus sp 0BB22S0, Synechococcus*, Synechococcus sp CCMP839, Cyanobium sp LB03, Synechococcus sp 0BB26S03, Synechococcus‡,* Chroococcaceae†, Chroococcales", and Cyanobacteria'.

The phylogeny of *Synechococcus* has been defined over the years and can be separated into five or six different clusters based on traits[43]. The role of these species in terms of the community is primary production and carbon fixation[28, 43, 58]. Researchers believe that these species are a major food source for protozooplankton and can fix atmospheric nitrogen[8, 29].

Notably, *Synechococcus sp.* are often found in association with *Prochlorococcus* in marine habitats, but the data here reveal no *Prochlorococcus sp*[7, 29, 43, 54]. Both Synechococcus and Prochlorococcus belong to the same clade as described by Urbach *et al*., which consists of both marine and freshwater cyanobacteria. Within this clade Synechococcus can be divided to a series of clusters, in which marine and freshwater species are separated[54]. It is probable that the freshwater strains of Synechococcus identified for this study are part of the Synechococcus cluster that dominates freshwater systems and outcompetes Prochloroccus strains[28 43, 54].

ACTINOBACTERIA: OHIO RIVER.

Actinobacteria, commonly known as high-G+C gram-positive bacteria, was the second most dominant phylum of the community, comprising 18-33% of the total. Members of this phylum are found in freshwater, terrestrial and marine environments, where their roles involve recycling organic matter[15, 19, 56].

Morphologically, Actinobacteria exhibit a wide variety in cell shape, from coccoid and rod-coccoid to hyphal forms and branched mycelium[56]. They have diverse physiological and metabolic properties as well, which include the production of extracellular enzymes and secondary metabolites[56].

At the lowest taxonomical level identified, the dominant Actinobacteria found for the Ohio River include: Actinomycetales", Microbacteriaceae†, Actinobacteria', Sanguibacteraceae†, Micrococcaceae†, Streptomycetaceae†, and Dermabacteraceae†. The fact that the listed Actinobacteria are not identified at the species level is due to the difficulty of isolating specific freshwater strains[16, 19]. The majority of classified aquatic Actinobacteria have been recovered from marine environments, therefore freshwater studies using culture-independent techniques can only make comparisons to these marine cousins[16, 19].

Although the dominant Actinobacteria found for the Ohio River cannot be recognized past the family level, all of the above listed do belong to the order Actinomycetales[50, 63]. In the soil, members of this order cycle carbon from organic debris. Other members of this order are important in the production of antibiotics, especially those in the Streptomycetaceae family[56].

PROTEOBACTERIA: OHIO RIVER.

Proteobacteria, or purple photosynthetic bacteria and relatives[49], were found to be the third most dominant phylum comprising 13.57-24.67% of the total Ohio River's bacterial community. This phylum accounts for the vast majority of the known gram-negative bacteria and vary in physiological characteristics including

phototrophs, heterotrophs, and chemolithotrophs[18]. The role of Proteobacteria in a community can vary among the subphyla Alpha, Beta, and Gamma. It is suggested that Alpha- and Gammaproteobacteria in combination with Bacteroidetes are responsible for the uptake and mineralization of dissolved organic matter in aquatic ecosystems[39]. Betaproteobacteria subphyla are comprised of freshwater ammonia-oxidizing bacteria and are often closely related to Gammaproteobacteria, which are found in higher percentages in polluted waters[44].

At the species level, or lowest taxonomical level classified, the dominant Proteobacteria found for the community consist of Rickettsiaceae†, Rhizobiales", and Alphaproteobacteria' within the subhphyla Alphaproteobacteria; Burkholderiaceae†, *Methylovorus‡, Cupriavidus‡, Polynucleobacter necessarius, Curvibacter gracilis, Curvibacter‡,* Comamonadaceae†, *Malikia‡,* Burkholderiales"*, Ramlibacter‡,* and *Polynucleobacter‡* within the subphyla Betaproteobacteria; and *Polyangiaceae†* within Deltaproteobacteria.

From the studies done on brackish and oceanic waters and on the Amazon River, Proteobacteria were found to be the dominant phylum, representing more than half of the community at some locations[15, 53]. Further identification of the sequences found in the Amazon River produced two species, *Polynucelobacter sp.* and *Streptomyces sp.,* which are similar to the dominant Proteobacteria and Actinobacteria species recovered from the Ohio River[15]. Matcher 's *et al.* study on the Eastern Cape estuary found Proteobacteria to be the dominant phylum with the highest abundance of species from the order Rhodobacterales and Rickettsiales[39]. This is similar to the Ohio River in that the dominant Proteobacteria was found to be

Rickettsiaceae†. Notably, the family Rickettsiaceae contains species that are known to be sensitive to environmental conditions, and are thus labeled as obligate intracellular pathogens. Since it is an obligate intracellular pathogen, it is probable that the Rickettsiaceae† found for this study was living in symbiosis with protists.

BACTERIAL COMMUNITY: GUYANDOTTE.

The Guyandotte River is a tributary of the Ohio River and is approximately 166 miles long spanning across several counties of southwestern West Virginia. The Guyandotte sampling site in particular reveals a diverse community very different from that of the Ohio River. The bacterial community at sampling site GUY is made up primarily of Proteobacteria (80.72%) followed by Actinobacteria (12.45%), Bacteroidetes (3.81%) and OP10 (1.03%). Cyanobacteria are largely missing from the community with the dominant Cyanobacteria species only making up 0.13% of the community. This lack of cyanobacteria could be explained by the high turbidity found at the sampling site affecting the availability of light for the organisms to thrive. Overall it is to be expected that the amount of cyanobacteria would be different from the Ohio River due to the Guyandotte's size and surrounding environment.

PROTEOBACTERIA & ACTINOBACTERIA: GUYANDOTTE.

The majority of the Proteobacteria and Actinobacteria found at the Guyandotte sampling site are different from those found in the Ohio River. The dominant species of the classified Proteobacteria for the Guyandotte was found to be *Curvibacter‡* (21.73%) followed by Comamonadaceae† (14.53%). The species could not be

identified for *Curvibacter‡* nor could the genus for Comamonadaceae†, but both belong under the subphyla Betaproteobacteria. As mentioned before, Betaproteobacteria are freshwater ammonia-oxidizing bacteria that are often found in higher percentages in polluted waters[44]. Comamonadaceae are described as gram-negative chemoorganotrophic or chemolithotrophic bacteria, which use oxygen or nitrates as their terminal electron acceptor[59]. This family contains the genera Hydrogenophaga‡, which was found at 1.19% of the Guyandotte community and the species *Hydrogenophaga atypica* at 1.26%.

The dominant Actinobacteria for the Guyandotte are Actinomycetales" (3.14%) followed by Streptomycetaceae† (2.62%), which are predominantly soil bacteria that cycle carbon from debris and are known for producing antibiotics[56]. A soil bacterium in the Guyandotte is not a surprising result considering the high turbidity of the Guyandotte on the day of sampling.

WATER QUALITY: GUYANDOTTE.

A reason the Guyandotte's community is different from the Ohio River can be found in the water quality data (Table 1) and from other environmental factors such as weather and pollution. The water quality data show that the water temperature and turbidity is drastically different in comparison to the other sampling sites. For instance, the water temperature within the Ohio River was around 30˚C while the Guyandotte's water temperature was recorded at 25.58˚C. The turbidity of the Guyandotte—being at 134.7ntu in comparison to the Ohio River's at an average of 13ntu—and the flow of the Guyandotte on the average day, suggests the possibility

that a disturbance of some kind affected the natural bacterial community. This could be due to heavy rainfall, yet the National Weather Service has no record of any rainfall near Huntington or the surrounding counties that could affect the Guyandotte's turbidity. Therefore, it is unknown as to why the Guyandotte was so heavily turbid on the day of sampling.

According to Thompsons *et al.*, biochemical changes from one sampling location to another could allow microbial communities to drastically change the dominant groups within[53]. The changes that made an impact on Thompsons *et al.* study were soil and temperature. In a site where soil was carried in the water, the most endemic, or unique to that site, taxa included an abundance of soil microbes[53]. This data strongly suggests that soil microbes and freshwater microbes do often overlap within a community due to the interactions between the two environments[7]. Where the unique community obtained from the Guyandotte originates from and if the species are present on a typical day needs to be further researched, but the high turbidity and lower temperature do suggest biochemical influence.

The above conditions could explain the lack of Cyanobacteria present. As turbid as the water was, very little light could penetrate the benthic layer, which would inhibit the growth of Cyanobacteria. In Thompsons *et al.* study, Cyanobacteria were low in abundance since the sample collected for the study was rather turbid, inhibiting the growth of this phylum[15, 53]. In addition, Betaproteobacteria have the ability to adapt to different environmental conditions, including low-nutrient concentrations, and are often able to dominate in conditions where other taxa cannot[44]. Considering the dominant phyla found for the Guyandotte was

Proteobacteria at 80.72%, there is a high probability of decreased nutrient availability when conditions are not optimal.

In terms of influence on the Ohio River, it does not seem likely that the Guyandotte on an average day has much effect on the bacterial community present in the Ohio River. This could be attributed to the flow of the Ohio River being on average significantly higher than the Guyandotte's. When looking at Tables 4 & 5, the list of dominant species present at each sampling site, it is observed that the dominant species found in the Guyandotte are not present in a large percentage in the Ohio River. If any influence from the Guyandotte on the Ohio River did manifest itself, it would be downstream from the mouth of the Guyandotte. However, the sampling site DS GUY, downstream in the Ohio River from the entry point of the Guyandotte into the river, shows that the community present there still resembles the community found upstream of the Ohio River, with a PS= 90.46% from the OH sampling site (Table 10). The possibility exists, however, that a unique species from the Guyandotte not found at that point in the Ohio, could be introduced here and could become numerically relevant downstream.

SPECIES RICHNESS AND DIVERSITY.

The bacterial diversity and species richness found for the Guyandotte is different from the Ohio River's. The species richness for sampling site GUY shows a high species richness at a value of 286 with 186 different taxa unique to the site itself (Table 6.) Local species richness can be influenced by many factors, such as disturbance to the habitat. If the habitat has been disturbed, species richness could

increase due to less competition or through the introduction of more species to the habitat. The latter could explain the high species richness in the Guyandotte.

According to Thompsons *et al.* study in which pyrosequencing was used on brackish and oceanic waters, the common sequences between each location showed that all sites shared approximately 0.6% of their microbiota[53]. This suggests a microbial dispersion or wide distribution. From this study's data, the sites along the Ohio River share 66.48% similarity between each other and 15.12% similarity between the Guyandotte. When each site was compared to each other, the proportional similarity showed the downstream site (DS GUY) being 90.42% similar to the upstream OH site. For comparison of across the river, the OH site and WV site showed a 94.92% similarity (Table 10). This suggests no significant difference between the upstream and downstream sites in terms of community structure.

The overall proportional similarity (66.48%) between the Ohio River sites could be attributed to the fact that sites OH and WV had only one sample taken whereas the other sites had two samples and site CH having three. The number of replicates at each site affected the species richness, as the sites with more samples had more species (n) taken into account. For instance, between the OH site upstream and site OH GUY downstream, n= 187 compared to n= 233, with species richness S= 102 versus S= 109. The species richness for site CH with three replicates had n= 239 and S= 111, which is within the same range as the sites with two samples. The number of unique/rare (k) species at site OH and WV (k= 2) were lower than site CH and the downstream sites as well (k= 9-12).

Among the replicates, site CH (which had three samples) had a 92.04% similarity, site GUY had 82.97% similarity, site CH GUY had 92.04%, site OH GUY had 92.22%, and the last site DS GUY has 92.90% similarity between its two samples. The question raised from these results is what should be considered to be a replicate for further studies. It could be inferred that more replicates would be helpful in determining species richness and diversity, however, the similarities among the replicates suggest each sample taken at a given site captured a good portion of the community present. It is probable that sites OH and WV, which only had one sample taken, the community found there is accurate for those sites, but two replicates could yield a better community profile.

The Simpson's Diversity Index results show that the sites have high species evenness along with richness. With values ranging from 0 to 1, with 1 meaning all species in the community are equal in numbers, the sites range from 0.86 at site OH to 0.92 at site CH (Table 7). When comparing the upstream sites to the downstream sites, there is no significant difference between the communities. The only sites that show a significant difference among the species diversity is between site OH vs. GUY (Ds= 0.93) with a t-test value of -2.1, and site WV vs. GUY with a value of -2.0 on a scale where a value of 1.9 and higher is considered significant (Table 8).

Although the Simpson's Diversity Index for the Guyandotte is similar to the Ohio River's, the Guyandotte's community shows different phyla and species, which are supported by the statistical data shown by the t-test values, species richness, and proportional similarity. When comparing the Ohio River to the Guyandotte, there is only a 15.12% similarity. Therefore, it can be assumed that the bacterial community

in the Guyandotte at the time of sampling is structured in a different way than that of the Ohio River.

BACTERIAL COMMUNITY: CULTIVATION.

The data obtained from pyrosequencing on the cultivated bacteria reveals a different community than what was found from the Ohio River. Although the water used to inoculate the Petri dishes came from a sample from the Ohio River and was sequenced via the same technology, the bacteria community does not reflect the data found for that site. If the cultivated data were the only source available to understand the bacterial diversity of the Ohio River, the results would reveal a community made up of phyla Proteobacteria, Firmicutes, and Bacteroidetes/Chorobi bacteria with a small percentage of Actinobacteria and Cyanobacteria. A study done on two shallow lakes in eastern China used culture-dependent techniques to identify the bacterial communities and found an abundance of Proteobacteria (82%) with Firmicutes and Bacteroidetes at 4.5% of the total[61]. These phyla, including Actinobacteria and low-G+C gram-positive bacteria, are well represented by cultivation and therefore more readily identified[26].

CYANOBACTERIA: CULTIVATED.

One possibility as to why there were very little Cyanobacteria present on the plates is due to the method used for cultivation. According to Stanier's *et al.*, work on cultivating Cyanobacteria in the laboratory, when plating an environmental sample on mineral rich media then incubating under light at 35C°, the population would be exclusively Cyanobacteria. In addition, in order to grow Cyanobacteria in

the dark, the medium on which they are inoculated has to be rich in carbohydrates[51]. Growth in the dark will be slow as they undergo a natural respiratory metabolism with the tricarboxylic acid cycle, which cannot occur in the dark; therefore, switching to the glycolysis or pentose phosphate cycle is more energy expensive[51]. Stainer *et al.* also mentions that obtaining a pure culture of Cyanobacteria is difficult because most species often have a slime layer that traps other bacteria within it[51]. In terms of the dominant species found for the community as a whole, it is important to note that Stainer *et al.* found in his study that the genus *Synechococcus sp* never formed colonies or produced a slime layer. Since the cultures for this study were plated on non-selective R2A media and incubated in the dark at 30C, it is likely that the Cyanobacteria that were present did not grow properly to be detected during pyrosequencing[51].

PROTEOBACTERIA: CULTIVATED.

Of the dominant cultivated Proteobacteria species, *A. veronii* at 21.20%, and *Aeromonas schubertii* at 9.90% are known to be pathogenic to humans and can be cultured easily in the laboratory[22, 23]. Reported cases with *A. veronii* infections usually coincide with exposure to freshwater systems[22]. This strain of *Aeromonas* is also closely related to *A. jandaei,* of which the cultivated represented 4.22%, yet unlike *A. veronii, A. jandaei* is not a threat to humans but has been reported pathogenic to fish[11]. *Alishewanella‡* at 10.14% of the community is also potentially pathogenic to humans and found to be related to *Aeromonas*[57].

At 9.90% of the total Proteobacteria, the isolate *Vogesella‡* has been cultivated from freshwater and soil communities[17]. This genus, along with *Pseudomonas sps,* is particularly well known for its striking blue-purple and yellowish pigmentations and is easily grown on agar in the laboratory[17]. Since the majority of the cultivated was Proteobacteria and of the isolated strains, it is possible that strains 31311, 4317, and 4427 could belong to this genera based on their colony coloration.

FIRMICUTES & BACTEROIDETES/CHOROBI GROUP: CULTIVATED.

The dominant Firmicutes cultivated all belong under the *Bacillus* genus, with *Bacillus thuringiensis* at 5.50%, *Bacillus mycoides* (1.76%), *Bacillus weihenstephanensis* (0.93%), *Bacillus‡* (0.61%), and *Bacillus cereus* (0.49%) are all pathogenic to humans[3, 30, 34]. These *Bacillus sps.* can be found in habitats where temperatures often fluctuate diurnally and seasonally for they are adaptable to many niches[34]. All of the above have been well studied in cultures and in their roles as pathogens to humans.

As for the cultivated Bacteroidetes/Chorobi group, the dominant taxon classified is Flavobacteriaceae† at 1.10% of the community. This family is very diverse and can be found in freshwater and soil habitats and has the potential to be pathogenetic[4]. Since it was not further classified, it can only be assumed what role this isolate plays in terms of the community.

Although the majority of the cultivated species are pathogenic to humans, that does not necessarily mean the Ohio River is harmful. For example, *A. veronii*, *Alishewanella‡, A. schubertii,* and *A. jandaei* are less than 0.09% in the community

found for the site CH (Table 13). In addition, the Firmicutes that the cultivation results revealed comprises at the most 0.04% of the total within the Ohio River community (Table 2). The cultivated pathogenic species are not in high percentages in the Ohio River therefore their threat to humans is undetermined and further research is needed.

CULTIVATED VERSUS PYROSEQUENCING.

A comparison of the dominant phyla found at sampling site CH (Table 2) to the data from the cultivable community (Table 11) reveals different dominant phyla. Furthermore, an observation of Table 13 of the Proteobacteria found for both the cultivated and sampling site shows that the species are different percentages. For the cultivated, the dominant species of Proteobacteria was found to be *A. veronii* at 21.20% while the site revealed that species to be only 0.02% of the community. When looking at the dominant species for the sampling site, Rickettsiaceae† was found at 5.72% of the community while the cultivated showed no trace of Rickettsiaceae†. This data alone strongly suggests that relying on cultivation as the main form for identifying bacterial communities from an environmental sample would be greatly misleading. Although the pathogenic species are not in high percentages in the Ohio River, it cannot be concluded that they do not pose a threat to humans, given the environmental conditions could change.

POTENTIAL SYMBIOTIC RELATIONSHIPS.

Out of 13 morphologically different isolated strains, eight were found to be potentially symbiotic in nature. These eight strains could not be separated out further and when observed under light microscope, at least two or three different cell shapes were found. When reviewing Table 14, each strain is listed along with the different cell shapes found within them. Although a DNA library was made, the strains never underwent DNA extraction and identification. However, the question of whether or not these strains are indeed pure or perhaps potentially symbiotic in nature comes to the forefront.

Gram staining revealed at least three different cell shapes for each strain, with one strain having four. All cells were found to be gram-negative, with only strain 51418 having a gram-positive within it. This coincides with the pyrosequencing results revealing more Proteobacteria than other phyla, since all classified Proteobacteria are gram-negative. The underlining question is this: if these isolated colonies that were extracted from the original plates are indeed pure, meaning the strain is separate from all other strains, why are they comprised of different cells? The solution could be that the definition of a pure strain for this study is a mixture of different species that rely on the support of one another to grow in the laboratory. Since the strains were not further sequenced, it can only be assumed that the species within could be potentially symbiotic in nature as well.

CHAPTER 5.

CONCLUSIONS.

COMMUNITY STRUCTURE AND FUNCTION.

The main focus of this study was to sample the Ohio River to gain a snapshot of the bacterial community residing in the river. It was expected that the results obtained from this study could be used to start additional research projects.

Large river systems, such as the Ohio River, are generally heterotrophic due to the external inputs of matter, which increases the carbon uptake and the rate of bacterial production[32]. The interactions between autotrophs and heterotrophs are essential to carbon and nutrient cycling, and in systems where heterotrophic bacteria dominate the primary production rates are often high as well[32]. The exact role each species play in the community can only be assumed at this point as the majority of the bacteria found for the Ohio River are hard to culture, therefore making it difficult to determine their functions. Further metagenomic analysis of the Ohio River to examine functional genes may shed light on the specific role of bacteria in the community.

This study showed that Cyanobacteria were the dominant bacteria phylum present when these samples were taken. Cyanobacteria are primary producers, able to fix carbon and nitrogen, and are a food source of other organisms. Cyanobacteria and heterotrophic bacteria in aquatic habitats are actively preyed upon by protists. Protist metabolism distributes carbon and energy, while their excretion of nitrogen, phosphorus and trace metals throughout the ecosystem is a major source of regenerated nutrients[12, 45]. Protists that consume suspended freshwater bacteria are

a significant source of food for metazooplankton, which are prey for fish and other organisms in the ecosystem. This predator-prey dynamic in aquatic systems recycles the carbon and nutrients made available by the cyanobacteria and heterotrophic bacteria.

Actinobacteria were the dominant heterotrophic phylum present. Actinobacteria can be found in freshwater, terrestrial and marine environments where their roles involve recycling organic matter[15, 19, 56]. It is likely they play a similar role in the Ohio River ecosystem. The Actinobacteria found in this study could not be identified at the species level largely due to the difficulty of isolating specific freshwater strains[16, 19]. The majority of classified aquatic Actinobacteria have been recovered from marine environments, therefore freshwater studies using culture-independent techniques can only make comparisons to marine cousins[16, 19].

Proteobacteria made up a similar fraction of the Ohio as the Actinobacteria. Proteobacteria are very diverse and may play a variety of different roles within an ecosystem. Alphaproteobacteria are responsible for nitrate/nitrite cycling, are found in correlation with chlorophyll concentrations, and prefer organic matter[44, 62, 65]. Betaproteobacteria are opportunistic bacteria that adapt well to low nutrient concentrations, are often comprised of ammonia-oxidizing bacteria, and are common in both oligotrophic and polluted systems[44]. In this study, the dominant Proteobacteria were from the subphyla Alphaproteobacteria along with species from subphyla Betaproteobacteria in lower concentrations.

From these results, we begin to see a snapshot of the bacterial community of the Ohio River. With a large number of cyanobacteria, a significant amount of carbon is

being fixed into biomass. The Actinobacteria present in the Ohio River are likely to be recycling carbon as well, although their roles in freshwater communities are not fully understood. The Proteobacteria present in the Ohio River are cycling nitrogen and oxidizing ammonia.

OHIO RIVER VERSUS GUYANDOTTE.

An important aspect of the study is the similarity of the Ohio River, both within replicates, across the river, and downstream but also between the Guyandotte river. The similarity between the Ohio River's sites replicates is 66.48%. Within replicates, excluding OH and WV sites where only one sample was taken, the proportional similarity for each site exceeds 90%. This data along with species richness provides information about how accurate and reliable the community is within the same location. Even so, in the future at least two samples per site would be needed to provide a well-balanced profile of the community present. This was demonstrated by the differences in taxa and species richness.

The community profile across and downstream within the Ohio is very similar and is represented by the proportional similarity data. Between the upstream OH site and downstream OH GUY site there is 90.11% similarity. Between WV site and DS GUY there is 90.04% similarity. Across the river, between sites OH and WV there is a 94.92% similarity. The data presented in this study along with the overall proportional similarity between the Ohio sites indicates a well-mixed community. This data is suggesting that any influence from the Guyandotte on the Ohio River at the sampling location is minimal. Further studies are needed to determine if the

Guyandotte or any tributary along the Ohio River has an impact as the water flows downstream.

The difference between the Guyandotte and the Ohio River shows how different a community can be even within the same region. The community profile of the Guyandotte versus the Ohio River is striking in many ways, including species richness and proportional similarity. The overall similarity between the Ohio River and the Guyandotte is only 15.12%, with significant difference in species diversity at upstream sites OH and WV. Although the Simpson's Diversity values for the Guyandotte compared to the Ohio River's downstream sites were not significant, they are on the higher end of the scale. This data along with the dominant species classified for both sampling locations supports the idea that bacterial community composition can shift within a region. It will be interesting to determine in future studies how the bacterial community of the Ohio River changes as it receives other inputs as it flows downstream. In addition, it will be interesting to study how the bacterial community of the river changes over seasonal conditions.

CULTIVATION.

From the cultivation results, the main point to be understood is that what can be easily cultivated in the laboratory does not resemble the true community from which it originates. Especially concerning at the potential impacts on humans, the cultivated species are well known because of their pathogenesis but are found in low numbers in the overall community. To determine if the pathogenic species are a health risk would require information about each species inoculation load to cause

disease. In addition, further sampling of the river is needed to track the numbers of these species for increases of decreases over seasonal or weather related changes to the environment that can have an affect on human contraction.

FUTURE STUDIES.

Future studies can build on the knowledge gained from this pilot project; particularly when more samples from upstream and downstream of the Ohio River can be collected and investigated. The amount of data that would be collected and consolidated into one long-term study could provide a complete picture of the bacterial diversity of the river. Furthermore, a larger scale project of the whole microbial community including viruses and other microbial organisms could be established using techniques such as gene-centric or genome-centric metagenomics. Studies have found that differences in community composition and functional genes along an environmental gradient have the potential of indicating human or natural impacts on a given ecosystem[60].

Future studies involving gene or genome-centric metagenomics could also help determine whether the given ecosystem is eutrophic or oligotrophic by searching for specific genes that can process nitrogen or phosphorus. Understanding bacterial diversity in terms of function within a community is essential when determining the overall health of an ecosystem. Further research is needed on the Ohio River's bacterial diversity to draw definitive conclusions about what the community found in this study is saying about the overall health of the Ohio River.

Cultivation has been the foundation of microbiology studies and has provided invaluable knowledge about the microbial world. Although next-generation sequencing techniques can now classify previously uncultivable and unknown bacteria, cultivation is still essential for future research when trying to understand physiological diversity and environmental roles in a community[26]. The combination of old and new classification and sequencing techniques has the potential to reveal all of the mysteries held within the field of microbiology.

The most important future research would be the continuation of classifying the Ohio River's bacterial diversity using next-generation sequencing techniques. It is essential to gather more data on the bacterial community residing in the river to compare and contrast the differences. Bacterial communities are in constant flux and documentation of these changes along gradients, seasons, and locations is needed to grasp their true population and role within the Ohio River's ecosystem.

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APPENDIX

RAW DATA: PYROSEQUENCING

Table 1.

Name	OH 1	WV 2	CH 3	CH4	CH 5	GUY 6	GUY 7	GUY CH 8	GUY CH 9	OH GUY 10	OH GUY 11	DS GUY 12	DS GUY 13
Cyanobacteria	65.38	64.86	20.51	41.76	53.71	0.58	0.28	62.50	58.40	58.74	56.33	64.51	64.28
Actinobacteria	18.66	18.26	42.66	31.66	23.96	15.61	9.28	19.42	22.94	20.75	21.95	19.75	20.50
Proteobacteria	14.05	14.66	31.95	22.93	19.14	76.47	84.96	16.13	16.20	17.65	19.51	13.70	13.43
Chloroflexi	0.59	0.60	0.91	0.82	0.98	0.22	0.17	0.52	0.65	0.73	0.65	0.48	0.50
Bacteroidetes	0.55	0.80	1.97	1.18	0.96	4.24	3.38	0.55	0.97	0.96	0.64	0.94	0.67
Verrucomicrobia	0.46	0.50	1.53	1.27	0.83	0.83	0.61	0.55	0.42	0.72	0.54	0.29	0.28
OP10	0.15	0.06	0.11	0.08	0.10	1.25	0.80	0.09	0.05	0.12	0.06	0.04	0.10
Gemmatimonadetes	0.11	0.16	0.26	0.26	0.24	0.12	0.10	0.12	0.18	0.20	0.12	0.14	0.13
WS3	0.02	0.07	0.01	0.01	0.02	0.01	0.03	0.05	0.04	0.05	0.02	0.06	0.06
Acidobacteria	0.02	0.00	0.00	0.00	0.00	0.11	0.05	0.01	0.00	0.01	0.02	0.01	0.00
TM7	0.00	0.00	0.00	0.00	0.00	0.31	0.16	0.00	0.00	0.00	0.00	0.00	0.01
Spirochaetes	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.01	0.00	0.00	0.01	0.01	0.01
Planctomycetes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
OD1	0.00	0.00	0.00	0.00	0.00	0.07	0.01	0.00	0.00	0.00	0.00	0.00	0.01
Nitrospirae	0.00	0.00	0.03	0.00	0.01	0.06	0.05	0.01	0.00	0.01	0.00	0.00	0.01
Fusobacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Firmicutes	0.00	0.03	0.01	0.01	0.03	0.04	0.08	0.01	0.07	0.00	0.08	0.04	0.00
Cercozoa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Candidatus Poribacteria	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
BRC1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Bacteria incertae sedis	0.00	0.02	0.04	0.01	0.00	0.03	0.01	0.02	0.09	0.04	0.06	0.03	0.02

Phyla Percentages for Ohio River and Guyandotte Sampling Sites. OH=Ohio bank, CH= Channel, WV= West Virginia bank, GUY= Guyandotte River, CH GUY= Ohio River Channel at the mouth of the Guyandotte, OH GUY= Ohio bank across from mouth of the Guyandotte, and DS GUY= Ohio River downstream from Guyandotte. Number after each site refers to sampling bottle.

Table 2.

Name	CH 3C	CH 4C	CH 5C
Cyanobacteria	0.00	0.00	0.01
Actinobacteria	0.05	0.03	0.25
Proteobacteria	92.39	92.70	80.38
Chloroflexi	0.00	0.00	0.00
Bacteroidetes	2.31	1.34	0.96
Verrucomicrobia	0.00	0.00	0.01
OP10	0.00	0.00	0.01
Gemmatimonadetes	0.00	0.00	0.00
WS3	0.00	0.00	0.00
Acidobacteria	0.00	0.00	0.00
TM7	0.00	0.00	0.00
Spirochaetes	0.00	0.00	0.00
Planctomycetes	0.00	0.00	0.00
OD1	0.00	0.00	0.00
Nitrospirae	0.00	0.00	0.00
Fusobacteria	0.00	0.00	0.00
Firmicutes	5.25	5.92	18.38
Cercozoa	0.00	0.00	0.00
Candidatus Poribacteria	0.00	0.00	0.00
BRC1	0.00	0.00	0.00
Bacteria incertae sedis	0.00	0.00	0.00

Phyla Percentages for Cultivated samples.

Table 3.

Name	OH 1	WV 2	CH 3	CH4	CH 5	GUY 6	GUY 7	GUY CH 8	GUY CH 9	OH GUY 10	OH GUY 11	DS GUY 12	DS GUY 13
Synechococcus sp 0BB22S0	35.72	35.43	11.11	26.68	32.03	0.20	0.06	35.42	32.69	31.38	31.07	31.69	28.83
Actinomycetales (unk family)	6.32	6.18	16.49	11.57	8.53	0.03	0.01	8.04	8.80	9.04	8.16	10.22	12.19
Microbacteriaceae (unk genus)	5.55	5.71	11.47	8.90	6.88	3.95	2.33	6.98	8.43	8.00	7.86	7.33	7.45
Synechococcus uncultured	9.66	9.02	2.37	3.50	6.28	1.72	1.16	6.08	6.44	5.75	6.75	5.78	6.14
Rickettsiaceae (unk genus)	2.77	3.31	8.18	5.09	3.89	0.02	0.00	3.61	3.25	4.99	3.60	5.33	5.59
Alphaproteobacteria (unk order)	1.30	1.62	1.65	3.17	2.61	0.06	0.00	2.74	2.63	4.18	4.43	4.72	5.04
Synechococcus sp CCMP839	4.05	4.31	10.48	7.31	5.86	2.09	1.24	4.34	5.22	4.58	4.94	4.18	4.35
Synechococcus sp 0BB26S03	3.34	3.38	0.59	1.84	2.23	0.59	0.28	2.47	2.61	2.27	3.57	3.89	3.11
Bacillariophyta uncultured	1.49	1.34	3.40	2.68	2.10	0.03	0.01	1.38	1.20	1.73	2.13	2.59	2.98
Actinobacteria (class) (unk order)	4.42	4.10	1.10	1.79	2.57	0.18	0.15	3.38	3.32	3.82	3.05	2.33	2.63
Rhizobiales (unk family)	1.96	2.82	5.52	4.13	3.33	0.02	0.00	3.64	3.21	2.32	2.82	2.10	1.74
Synechococcus (unk species)	1.86	2.24	0.56	0.85	1.61	0.40	0.18	1.75	1.98	1.12	1.36	1.23	1.25
Polyangiaceae (unk genus)	0.86	0.72	2.40	1.77	1.30	0.02	0.01	1.08	0.83	1.04	0.92	1.08	1.11
Cyanobium sp LB03	4.05	4.25	0.91	1.18	2.19	0.01	0.01	2.21	2.21	2.00	1.91	0.89	0.99
Chroococcaceae (unk genus)	1.04	0.94	0.46	0.50	0.86	0.58	0.28	0.76	1.04	0.89	0.79	0.56	0.73

Micrococcaceae (unk genus)	0.26	0.31	1.16	0.98	0.63	2.33	1.67	0.55	0.55	0.44	0.61	0.55	0.53
Synechococcus sp LBB3	0.18	0.20	0.33	0.35	0.31	1.42	1.15	0.30	0.56	0.65	0.64	0.67	0.51
Chroococcales (unk family)	0.57	0.64	0.96	0.76	0.54	0.79	0.51	0.36	0.35	0.27	0.39	0.54	0.49
Burkholderiaceae (unk genus)	0.53	0.49	1.14	0.84	0.63	0.58	0.38	0.50	0.53	0.39	0.35	0.55	0.45
Comamonadaceae (unk genus)	0.33	0.34	0.60	0.46	0.51	0.64	0.39	0.41	0.46	0.57	0.53	0.33	0.44
Streptomycetaceae (unk genus)	0.39	0.36	0.64	0.48	0.28	0.40	0.43	0.34	0.56	0.32	0.66	0.52	0.41
Bacteroidetes (unk class)	0.03	0.02	0.06	0.05	0.06	0.12	0.06	0.09	0.09	0.11	0.21	0.29	0.41
Caldilineales (unk family)	0.44	0.47	1.78	0.93	0.72	0.01	0.01	0.58	0.39	0.41	0.55	0.43	0.40
Proteobacteria (unk class)	0.37	0.33	0.66	0.47	0.47	3.38	1.86	0.27	0.37	0.54	0.45	0.49	0.40
Bacillariophyta uncultured(notFound)	0.24	0.30	0.28	0.34	0.40	21.74	21.71	0.34	0.23	0.24	0.27	0.36	0.40
Cyanobacteria (class) (unk order)	0.38	0.51	0.72	0.39	0.46	0.19	0.28	0.29	0.37	0.39	0.51	0.39	0.38
Acetobacteraceae (unk genus)	0.06	0.08	0.21	0.19	0.17	0.12	0.10	0.21	0.25	0.54	0.28	0.47	0.36
Merismopedia (unk species)	0.21	0.22	0.14	0.27	0.26	0.01	0.01	0.35	0.39	0.29	0.39	0.20	0.35
Methylovorus (unk species)	0.45	0.33	0.64	0.35	0.33	0.19	0.12	0.19	0.41	0.32	0.44	0.48	0.31
Methylosarcina (unk species)	0.01	0.00	0.03	0.06	0.03	0.09	0.06	0.09	0.11	0.23	0.28	0.28	0.31
Betaproteobacteria (unk order)	0.16	0.08	0.39	0.27	0.20	0.48	0.27	0.19	0.21	0.33	0.34	0.41	0.30
Merismopedia tenuissima 0BB46S01	0.26	0.24	0.71	0.92	0.46	0.02	0.04	0.76	0.37	0.32	0.29	0.24	0.30
Sphingobacteriales (unk family)	0.29	0.39	0.13	0.33	0.43	12.30	16.76	0.48	0.41	0.22	0.52	0.36	0.28

Opitutaceae (unk genus)	0.32	0.43	0.72	0.66	0.78	0.89	0.44	0.44	0.65	0.55	0.49	0.39	0.27
Sphingobacteria (unk order)	0.09	0.15	0.46	0.32	0.30	0.00	0.00	0.23	0.19	0.21	0.13	0.27	0.27
Rickettsiales (unk family)	0.11	0.28	0.63	0.43	0.34	0.00	0.01	0.29	0.25	0.23	0.20	0.18	0.27
Sanguibacteraceae (unk genus)	0.57	0.53	0.20	0.19	0.46	7.87	7.83	0.35	0.18	0.12	0.12	0.23	0.25
Dermabacteraceae (unk genus)	0.25	0.15	0.28	0.29	0.24	1.17	0.63	0.19	0.16	0.09	0.27	0.21	0.25
Polynucleobacter necessarius	0.34	0.25	1.03	0.68	0.45	2.59	2.03	0.23	0.32	0.49	0.36	0.22	0.23
Flavobacteria (unk order)	0.01	0.01	0.00	0.01	0.02	0.08	0.04	0.05	0.09	0.13	0.10	0.20	0.21
Malikia granosa	0.01	0.03	0.01	0.00	0.01	0.03	0.00	0.02	0.07	0.09	0.14	0.12	0.20
Curvibacter (unk species)	0.32	0.23	0.24	0.29	0.22	0.00	0.00	0.31	0.23	0.15	0.26	0.21	0.19
Moraxellaceae (unk genus)	0.00	0.00	0.00	0.00	0.01	0.17	0.10	0.02	0.04	0.05	0.14	0.13	0.19
Beijerinckiaceae (unk genus)	0.05	0.07	0.11	0.14	0.22	0.01	0.02	0.22	0.11	0.09	0.14	0.23	0.18
Caldilineaceae (unk genus)	0.08	0.11	0.40	0.25	0.18	0.24	0.69	0.08	0.14	0.23	0.14	0.20	0.18
Bacteria (unk phylum)	0.49	0.52	0.70	0.49	0.48	0.07	0.07	0.40	0.48	0.49	0.44	0.17	0.18
Polynucleobacter (unk species)	0.08	0.07	0.10	0.13	0.19	1.53	1.23	0.26	0.18	0.23	0.14	0.11	0.18
Hahellaceae (unk genus)	0.00	0.02	0.13	0.14	0.14	0.29	0.22	0.04	0.07	0.10	0.12	0.12	0.17
Oscillatoriaceae (unk genus)	0.13	0.17	0.24	0.24	0.22	2.61	2.36	0.29	0.35	0.18	0.19	0.10	0.17
Legionellaceae (unk genus)	0.03	0.03	0.04	0.05	0.08	0.01	0.03	0.06	0.12	0.17	0.14	0.24	0.16
Deltaproteobacteria (unk order)	0.10	0.08	0.45	0.26	0.19	1.26	1.56	0.18	0.14	0.23	0.14	0.19	0.16
Aphanothece sp 0BB21S01	0.14	0.12	0.03	0.05	0.15	0.56	0.42	0.09	0.14	0.16	0.13	0.14	0.16
Noctuoidea (unk genus)	0.23	0.13	0.35	0.25	0.20	0.29	0.12	0.12	0.16	0.16	0.11	0.09	0.16

Spirulina (unk species)	0.01	0.06	0.13	0.10	0.09	0.82	0.88	0.12	0.09	0.11	0.07	0.12	0.15
Pseudomonadaceae (unk genus)	0.03	0.04	0.08	0.07	0.09	0.00	0.00	0.05	0.11	0.09	0.08	0.09	0.15
Nostocales (unk family)	0.01	0.00	0.00	0.00	0.01	0.10	0.08	0.02	0.04	0.02	0.05	0.02	0.14
Cupriavidus (unk species)	0.47	0.40	0.50	0.60	0.42	0.59	0.30	0.43	0.32	0.28	0.23	0.14	0.13
Herbaspirillum lusitanum	0.07	0.07	0.06	0.01	0.11	0.17	0.08	0.14	0.19	0.17	0.09	0.12	0.13
Burkholderiales (unk family)	0.18	0.10	0.26	0.22	0.15	0.01	0.02	0.23	0.18	0.15	0.14	0.20	0.12
Kineosporiaceae (unk genus)	0.09	0.12	0.15	0.16	0.17	0.00	0.00	0.14	0.05	0.16	0.10	0.13	0.12
Bradyrhizobiaceae (unk genus)	0.01	0.07	0.08	0.07	0.06	0.12	0.06	0.05	0.04	0.07	0.08	0.08	0.12
Rhodoferax (unk species)	0.02	0.01	0.01	0.02	0.01	0.00	0.00	0.03	0.05	0.05	0.10	0.12	0.11
Intrasporangiaceae (unk genus)	0.23	0.20	0.21	0.19	0.18	0.01	0.00	0.16	0.23	0.13	0.19	0.11	0.11
Cyanobium (unk species)	0.28	0.18	0.08	0.21	0.17	0.00	0.00	0.07	0.04	0.06	0.12	0.09	0.11
Helicobacteraceae (unk genus)	0.00	0.00	0.00	0.01	0.03	0.17	0.08	0.02	0.07	0.10	0.04	0.14	0.10
Herbaspirillum (unk species)	0.21	0.11	0.06	0.07	0.09	0.00	0.02	0.01	0.05	0.05	0.04	0.13	0.10
Clostridiales (unk family)	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.05	0.09	0.09	0.07	0.10
Caulobacterales (unk family)	0.07	0.06	0.13	0.09	0.11	0.85	0.71	0.09	0.09	0.18	0.08	0.03	0.10
Rhodoferax ferrireducens	0.01	0.01	0.00	0.00	0.01	0.22	1.15	0.02	0.00	0.01	0.04	0.01	0.10
Vogesella (unk species)	0.03	0.11	0.03	0.03	0.03	0.34	0.89	0.06	0.05	0.12	0.12	0.18	0.09
Microcystis aeruginosa NIES-298	0.02	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.02	0.02	0.01	0.03	0.09
Anabaena (unk species)	0.01	0.04	0.13	0.08	0.03	0.00	0.09	0.01	0.00	0.00	0.01	0.04	0.08
Comamonas (unk species)	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.07	0.09	0.07
Schlegelella (unk species)	0.12	0.13	0.18	0.18	0.19	0.42	0.51	0.17	0.07	0.02	0.08	0.05	0.07
Magnetospirillum magneticum	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.03	0.02	0.07

Novosphingobium stygium	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.02	0.07
Microcystis wesenbergii	0.00	0.00	0.00	0.01	0.00	0.08	0.01	0.00	0.00	0.00	0.01	0.01	0.07
Pseudomonas luteola	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.02	0.09	0.06
Microcystis holsatica	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.02	0.05	0.06
Methylophilaceae (unk genus)	0.11	0.06	0.06	0.08	0.08	0.00	0.02	0.05	0.11	0.06	0.06	0.14	0.05
Vogesella indigofera	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.02	0.07	0.05
Opitutus (unk species)	0.10	0.03	0.06	0.03	0.03	0.04	0.08	0.02	0.02	0.00	0.02	0.04	0.05
Curvibacter gracilis	0.36	0.24	0.08	0.23	0.22	0.10	0.08	0.17	0.19	0.13	0.11	0.04	0.05
Oscillatoriales (unk family)	0.02	0.05	0.01	0.02	0.03	0.11	0.02	0.05	0.09	0.04	0.08	0.04	0.05
Cupriavidus metallidurans	0.00	0.01	0.04	0.00	0.01	0.02	0.00	0.01	0.00	0.00	0.00	0.04	0.05
Phenylobacterium (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.04	0.04	0.03	0.05
Chromatiaceae (unk genus)	0.00	0.00	0.00	0.00	0.01	0.07	0.03	0.00	0.02	0.07	0.03	0.03	0.05
Cyanobacteria (order) (unk family)	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.05	0.09	0.09	0.02	0.05
Thalassiosira eccentrica	0.07	0.07	0.04	0.13	0.11	0.04	0.01	0.04	0.05	0.04	0.02	0.00	0.05
Fluviicola (unk species)	0.00	0.00	0.03	0.02	0.02	0.11	0.11	0.01	0.00	0.00	0.00	0.00	0.05
Ktedonobacteraceae (unk genus)	0.00	0.00	0.04	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.07	0.04
Herbaspirillum chlorophenolicum	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.04	0.02	0.10	0.05	0.04
Rhodobacter sphaeroides	0.00	0.01	0.04	0.05	0.02	0.00	0.00	0.02	0.02	0.13	0.11	0.04	0.04
Jannaschia (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.04	0.04
Chroococcales (family) (unk genus)	0.32	0.29	0.15	0.09	0.21	0.35	0.25	0.16	0.14	0.12	0.05	0.02	0.04
Oceanospirillaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.15	0.12	0.00	0.02	0.00	0.04	0.02	0.04
Neisseriaceae (unk genus)	0.00	0.01	0.01	0.03	0.02	0.00	0.00	0.00	0.02	0.00	0.01	0.01	0.04

Rhizobium giardinii	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.01	0.04
Phyllobacterium (unk species)	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.03	0.00	0.05	0.05	0.00	0.04
Xanthomonadales (unk family)	0.03	0.03	0.00	0.02	0.06	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.04
Methylobacteriaceae (unk genus)	0.00	0.01	0.07	0.01	0.03	0.04	0.02	0.05	0.09	0.05	0.13	0.09	0.03
Brevibacterium (unk species)	0.00	0.00	0.00	0.00	0.00	0.06	0.07	0.01	0.00	0.00	0.00	0.06	0.03
Gemmatimonadetes (class) (unk order)	0.02	0.03	0.08	0.06	0.06	0.37	0.32	0.04	0.11	0.11	0.04	0.04	0.03
Actinobacteria (unk class)	0.30	0.25	0.08	0.06	0.12	0.07	0.01	0.07	0.00	0.01	0.02	0.04	0.03
Nostocaceae (unk genus)	0.04	0.07	0.13	0.13	0.18	0.00	0.00	0.12	0.09	0.18	0.07	0.04	0.03
Sphingomonadaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.10	0.13	0.03	0.00	0.04	0.04	0.04	0.03
Paenibacillaceae (unk genus)	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.03	0.03
Rhodospirillales (unk family)	0.04	0.11	0.00	0.01	0.02	0.03	0.00	0.02	0.00	0.01	0.02	0.03	0.03
Pseudomonas fulva	0.00	0.00	0.00	0.00	0.00	0.02	0.36	0.00	0.00	0.01	0.01	0.01	0.03
Alistipes putredinis	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.01	0.01	0.03
OP10 unclassified	0.02	0.01	0.03	0.03	0.02	0.06	0.01	0.00	0.00	0.00	0.00	0.01	0.03
Shewanella putrefaciens	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.02	0.00	0.03
Sphingomonas phyllosphaerae	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.04	0.05	0.02
Pelobacteraceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.05	0.02
Spirochaetaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.05	0.02
TM7 (genus) (unk species)	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.00	0.00	0.04	0.03	0.04	0.02
Xanthobacteraceae (unk	0.00	0.00	0.00	0.00	0.00	0.07	0.05	0.05	0.04	0.01	0.03	0.04	0.02

genus)													
Herbaspirillum seropedicae	0.21	0.14	0.08	0.03	0.09	0.34	0.11	0.07	0.11	0.16	0.11	0.04	0.02
Malikia (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.04	0.02
Leptothrix (unk species)	0.14	0.10	0.29	0.19	0.18	0.00	0.00	0.10	0.04	0.04	0.06	0.03	0.02
Ramlibacter (unk species)	0.14	0.13	0.20	0.11	0.09	0.75	0.91	0.04	0.07	0.01	0.03	0.03	0.02
Ktedonobacterales (unk family)	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.02
Microbacterium thalassium	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.02	0.02
Synechococcus sp LBG2	0.02	0.02	0.00	0.00	0.00	0.03	0.01	0.01	0.00	0.00	0.00	0.02	0.02
Solibacteraceae (unk genus)	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.05	0.05	0.04	0.01	0.02
Coscinodiscus radiatus(notFound)	0.02	0.01	0.00	0.00	0.02	0.19	0.44	0.02	0.07	0.09	0.03	0.01	0.02
Cryomorphaceae (unk genus)	0.01	0.03	0.00	0.00	0.09	0.00	0.00	0.04	0.05	0.02	0.03	0.01	0.02
Ktedonobacteria (unk order)	0.00	0.01	0.08	0.08	0.04	0.08	0.10	0.05	0.02	0.01	0.01	0.01	0.02
Hydrogenophaga (unk species)	0.00	0.02	0.00	0.00	0.00	0.44	0.22	0.01	0.02	0.01	0.01	0.01	0.02
Chryseobacterium gleum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.02
Methylophilales (unk family)	0.02	0.02	0.00	0.00	0.02	0.04	0.03	0.03	0.05	0.61	0.08	0.00	0.02
Thalassiosira pseudonana	0.06	0.07	0.03	0.03	0.03	0.00	0.00	0.07	0.07	0.02	0.03	0.00	0.02
Chromatiales (unk family)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.02
Chryseobacterium taiwanense	0.00	0.00	0.01	0.01	0.02	0.00	0.00	0.01	0.04	0.04	0.01	0.00	0.02
Micromonosporaceae (unk genus)	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02
Acidisphaera (unk species)	0.01	0.01	0.00	0.00	0.01	0.28	0.33	0.00	0.00	0.00	0.01	0.00	0.02

Serratia (unk species)	0.00	0.00	0.00	0.00	0.00	0.06	0.01	0.00	0.00	0.00	0.00	0.00	0.02
Nitrospira moscoviensis	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02
Burkholderia cenocepacia	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Acidobacteriaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Roseomonas (unk species)	0.03	0.06	0.07	0.08	0.09	0.08	0.03	0.09	0.12	0.18	0.05	0.04	0.01
Opitutae (unk order)	0.02	0.00	0.00	0.00	0.00	0.08	0.03	0.02	0.00	0.00	0.01	0.04	0.01
Gemmatimonadales (unk family)	0.09	0.12	0.01	0.00	0.04	0.17	0.14	0.04	0.09	0.10	0.06	0.03	0.01
Variovorax (unk species)	0.00	0.00	0.00	0.00	0.00	0.11	0.04	0.02	0.05	0.00	0.03	0.03	0.01
Synechococcus sp CC9311	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.01
Methylovorus sp	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.01
Verrucomicrobiales (unk family)	0.00	0.00	0.00	0.00	0.00	0.13	0.13	0.02	0.04	0.00	0.01	0.02	0.01
Nitrosomonadales (unk family)	0.00	0.00	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.01
Aquitalea (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01
Geobacter bemidjiensis	0.00	0.01	0.01	0.00	0.01	0.10	0.05	0.02	0.00	0.07	0.02	0.01	0.01
Methylobacterium radiotolerans	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.02	0.00	0.02	0.01	0.01
Caulobacter (unk species)	0.00	0.00	0.00	0.00	0.01	0.04	0.05	0.01	0.02	0.05	0.01	0.01	0.01
Burkholderia (unk species)	0.05	0.01	0.00	0.00	0.01	0.09	0.11	0.01	0.04	0.04	0.01	0.01	0.01
OD1 (class) (unk order)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.01	0.01
Burkholderia ambifaria	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.01	0.01
Pseudomonas fluorescens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.01
Legionella pneumophila	0.00	0.00	0.01	0.00	0.03	0.01	0.06	0.05	0.05	0.02	0.01	0.00	0.01
Methylosarcina lacus	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.02	0.02	0.01	0.00	0.01
Massilia (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.01
Arcobacter (unk species)	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.04	0.02	0.00	0.01	0.00	0.01
Pseudomonadales (unk family)	0.05	0.03	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.01

Alcaligenes faecalis	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.02	0.02	0.00	0.00	0.01
Anabaena solitaria BC Ana 0025	0.00	0.02	0.01	0.01	0.02	0.00	0.01	0.06	0.05	0.01	0.00	0.00	0.01
Legionella (unk species)	0.00	0.02	0.03	0.03	0.02	0.00	0.00	0.02	0.00	0.01	0.00	0.00	0.01
Flavobacterium (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01
Sphingobium herbicidovorans	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Sanguibacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.08	0.00	0.00	0.00	0.00	0.00	0.01
Rhodomicrobium sp	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Rhodobacteraceae (unk genus)	0.02	0.01	0.00	0.01	0.00	0.01	0.08	0.00	0.00	0.00	0.00	0.00	0.01
Pseudomonas putida	0.00	0.00	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Pseudomonas argentinensis	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Prosthecobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Propionivibrio limicola	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Pelobacter propionicus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Nephroselmis olivacea(notFound)	0.01	0.01	0.00	0.02	0.01	0.00	0.55	0.00	0.00	0.00	0.00	0.00	0.01
Magnetobacterium sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Krasilnikovia sp	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.01
Desulfuromonadales (unk family)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Desulfovibrio magneticus	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.01
Candidatus Cuticobacterium sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Azospirillum brasilense	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01
Micromonospora matsumotoense	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.05	0.10	0.03	0.09	0.00

Cryobacterium (unk species)	0.01	0.07	0.13	0.19	0.09	0.06	0.05	0.08	0.12	0.02	0.07	0.06	0.00
Methylocaldum (unk species)	0.00	0.01	0.11	0.05	0.04	0.00	0.00	0.03	0.02	0.00	0.01	0.03	0.00
Heterosigma akashiwo(notFound)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00
Oxalobacteraceae (unk genus)	0.04	0.05	0.03	0.01	0.02	0.46	0.66	0.01	0.02	0.00	0.00	0.03	0.00
Raphidiopsis curvata HB1	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.00
Synechococcus sp WH 7803	0.01	0.01	0.00	0.02	0.04	0.02	0.00	0.07	0.04	0.06	0.02	0.02	0.00
Hyphomicrobiaceae (unk genus)	0.20	0.11	0.15	0.25	0.20	0.00	0.00	0.12	0.05	0.02	0.02	0.02	0.00
Alishewanella (unk species)	0.03	0.05	0.10	0.01	0.03	0.24	0.19	0.00	0.00	0.00	0.02	0.02	0.00
Phoridium (unk species)	0.02	0.02	0.00	0.00	0.01	0.13	0.11	0.02	0.04	0.07	0.01	0.02	0.00
Pseudoxanthomonas (unk species)	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.01	0.02	0.00
Verrucomicrobiaceae (unk genus)	0.00	0.01	0.00	0.01	0.03	0.07	0.05	0.01	0.04	0.00	0.01	0.02	0.00
Solibacterales (unk family)	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.01	0.02	0.00
Cellvibrio (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.02	0.00
Levilinea (unk species)	0.01	0.00	0.00	0.00	0.00	0.07	0.02	0.00	0.02	0.00	0.00	0.02	0.00
Streptomyces lavendulae	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.02	0.00
Rubrivivax (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Rickettsia (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Erythrobacteraceae (unk genus)	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.00
Sphingopyxis alaskensis	0.00	0.01	0.00	0.00	0.00	0.45	0.31	0.00	0.04	0.01	0.01	0.01	0.00
Synechococcus sp WH 8101	0.12	0.06	0.17	0.02	0.12	0.06	0.07	0.07	0.02	0.01	0.01	0.01	0.00

Pleurocapsales (unk family)	0.03	0.01	0.03	0.00	0.01	0.08	0.03	0.03	0.02	0.01	0.01	0.01	0.00
OP10 (order) (unk family)	0.02	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.00	0.01	0.01	0.01	0.00
Thiotrichales (unk family)	0.00	0.00	0.00	0.00	0.00	0.07	0.02	0.00	0.00	0.00	0.01	0.01	0.00
Aeromonas punctata	0.02	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.01	0.00
Yersinia (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
TM7 (unk class)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Thauera (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.00	0.00	0.00	0.00	0.01	0.00
Sterolibacterium (unk species)	0.00	0.00	0.00	0.00	0.01	0.08	0.02	0.00	0.00	0.00	0.00	0.01	0.00
Rhodospirillaceae (unk genus)	0.00	0.01	0.06	0.01	0.00	0.03	0.27	0.00	0.00	0.00	0.00	0.01	0.00
Pseudomonas geniculata	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.00
Phyllobacterium trifolii	0.00	0.00	0.01	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.01	0.00
Phenylobacterium immobile	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Novosphingobium hassiacum	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
Nitrosomonadaceae (unk genus)	0.05	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
Microbacterium (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Mesorhizobium ciceri	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.01	0.00
Lysobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Hyphomicrobium (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
Cryocola (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Coxiellaceae (unk genus)	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
Coscinodiscus radiatus	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
BRC1 (order) (unk family)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
Beijerinckia derxii	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Beijerinckia (unk species)	0.11	0.07	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00

Bacillus mycoides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Bacillus licheniformis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Acidovorax temperans	0.00	0.00	0.00	0.00	0.00	0.07	0.07	0.00	0.00	0.00	0.00	0.01	0.00
Acidovorax avenae	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00
Rhodocyclaceae (unk genus)	0.08	0.06	0.04	0.01	0.03	0.11	0.16	0.02	0.02	0.04	0.06	0.00	0.00
Rhodobacterales (unk family)	0.04	0.05	0.03	0.03	0.04	0.00	0.00	0.03	0.09	0.05	0.04	0.00	0.00
Skeletonema costatum	0.04	0.03	0.08	0.07	0.02	0.00	0.02	0.02	0.02	0.01	0.03	0.00	0.00
Shewanella pealeana	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.00
Mitsuaria (unk species)	0.00	0.01	0.00	0.00	0.01	0.23	0.42	0.00	0.00	0.00	0.02	0.00	0.00
Hydrogenophaga pseudoflava	0.00	0.00	0.00	0.00	0.01	0.15	0.06	0.01	0.02	0.04	0.01	0.00	0.00
Bacteroidetes (class) (unk order)	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.02	0.00	0.04	0.01	0.00	0.00
OP10 (genus) (unk species)	0.00	0.01	0.00	0.00	0.03	0.11	0.12	0.00	0.04	0.02	0.01	0.00	0.00
Myxococcales (unk family)	0.02	0.07	0.00	0.02	0.03	0.00	0.17	0.02	0.02	0.02	0.01	0.00	0.00
Mycobacterium conceptionense	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.02	0.01	0.00	0.00
Sphingobacteriaceae (unk genus)	0.05	0.09	0.08	0.05	0.06	0.11	0.09	0.05	0.02	0.01	0.01	0.00	0.00
Ramlibacter tataouinensis	0.01	0.01	0.00	0.03	0.01	0.00	0.00	0.03	0.00	0.01	0.01	0.00	0.00
Sphingomonadales (unk family)	0.02	0.02	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00
Mycobacterium (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.01	0.01	0.00	0.00
Ensifer (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00
Achromobacter xylosoxidans	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.01	0.01	0.00	0.00
Paenibacillus chitinolyticus	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.03	0.07	0.00	0.01	0.00	0.00

Rhodobacter (unk species)	0.00	0.00	0.03	0.03	0.01	0.21	0.13	0.02	0.02	0.00	0.01	0.00	0.00
Alcaligenes (unk species)	0.00	0.00	0.00	0.00	0.01	0.04	0.04	0.05	0.00	0.00	0.01	0.00	0.00
Opitutales (unk family)	0.01	0.01	0.00	0.02	0.00	0.10	0.03	0.02	0.00	0.00	0.01	0.00	0.00
Pseudomonas otitidis	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.00	0.00	0.00	0.01	0.00	0.00
Mitsuaria chitosanitabida	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Haliscomenobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
Clostridium bifermentans	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.01	0.00	0.00
Bacillus weihenstephanensis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Actinosynnemataceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
Pseudomonas mendocina	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.00	0.00	0.00
Roseateles (unk species)	0.00	0.00	0.06	0.01	0.02	0.00	0.00	0.01	0.04	0.04	0.00	0.00	0.00
Cyanobium sp 0BB42S04	0.07	0.09	0.14	0.10	0.08	0.00	0.01	0.04	0.09	0.02	0.00	0.00	0.00
Chloroflexi (class) (unk order)	0.00	0.00	0.01	0.02	0.02	0.63	0.60	0.02	0.04	0.02	0.00	0.00	0.00
Sphingopyxis (unk species)	0.03	0.01	0.00	0.01	0.02	1.13	0.66	0.02	0.04	0.02	0.00	0.00	0.00
Cyanobacteria (unk class)	0.12	0.10	0.07	0.09	0.06	0.01	0.00	0.01	0.04	0.02	0.00	0.00	0.00
Streptomyces sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
Methylibium (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00
Candidatus Planktoluna (unk species)	0.01	0.02	0.03	0.03	0.03	0.04	0.03	0.05	0.05	0.01	0.00	0.00	0.00
Scherffelia dubia(notFound)	0.06	0.03	0.08	0.03	0.02	0.00	0.00	0.03	0.02	0.01	0.00	0.00	0.00
Microcystis aeruginosa NIES-843	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.01	0.00	0.00	0.00
Methylocapsa (unk species)	0.04	0.05	0.03	0.02	0.00	0.08	0.58	0.01	0.02	0.01	0.00	0.00	0.00

Flavobacteriales (unk family)	0.01	0.04	0.06	0.08	0.04	0.12	0.14	0.01	0.02	0.01	0.00	0.00	0.00
Thermomicrobiales (unk family)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.61	0.00	0.01	0.00	0.00	0.00
Oscillatoriales (family) (unk genus)	0.02	0.02	0.00	0.01	0.01	0.00	0.00	0.03	0.00	0.01	0.00	0.00	0.00
Methylocystaceae (unk genus)	0.05	0.02	0.04	0.00	0.01	0.25	0.28	0.02	0.00	0.01	0.00	0.00	0.00
Brucellaceae (unk genus)	0.01	0.01	0.01	0.00	0.01	0.20	0.12	0.02	0.00	0.01	0.00	0.00	0.00
Samsonia (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00
Rhizobium (unk species)	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00
Dechloromonas hortensis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00
Spirochaetales (unk family)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Solibacteres (unk order)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Shinella (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Serratia proteamaculans	0.00	0.00	0.00	0.00	0.01	0.08	0.14	0.00	0.00	0.01	0.00	0.00	0.00
Schlegelella thermodepolymerans	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Paracoccus carotinifaciens	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Novosphingobium pentaromativorans	0.00	0.00	0.01	0.00	0.01	0.00	0.08	0.00	0.00	0.01	0.00	0.00	0.00
Myxococcaceae (unk genus)	0.00	0.00	0.00	0.01	0.00	0.07	0.04	0.00	0.00	0.01	0.00	0.00	0.00
Mycobacterium pyrenivorans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Methylobacillus (unk species)	0.00	0.00	0.00	0.01	0.00	0.10	0.06	0.00	0.00	0.01	0.00	0.00	0.00
Levilinea sp	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Hydrogenophaga taeniospiralis	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Crocinitomix (unk	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00

species)													
Candidatus Rhodoluna (unk species)	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Candidatus Rhizobium sp	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Brucella (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Brevibacterium antiquum	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Bacteroidales (unk family)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Arthrobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Aeromonadaceae (unk genus)	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Achromobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Rhodobaca (unk species)	0.00	0.02	0.10	0.01	0.06	0.58	0.33	0.05	0.05	0.00	0.00	0.00	0.00
OD1 (unk class)	0.00	0.00	0.00	0.00	0.01	0.09	0.07	0.02	0.02	0.00	0.00	0.00	0.00
Aphanizomenon (unk species)	0.01	0.02	0.01	0.01	0.01	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00
Sphingopyxis witflariensis	0.00	0.01	0.00	0.00	0.00	0.01	0.02	0.01	0.02	0.00	0.00	0.00	0.00
Rhodopseudomonas palustris	0.00	0.00	0.04	0.01	0.06	0.06	0.06	0.01	0.02	0.00	0.00	0.00	0.00
Rhodocyclales (unk family)	0.00	0.03	0.00	0.00	0.06	0.07	0.06	0.01	0.02	0.00	0.00	0.00	0.00
Pseudomonas stutzeri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00
Bacillus thuringiensis	0.00	0.01	0.00	0.00	0.00	0.17	0.03	0.01	0.02	0.00	0.00	0.00	0.00
Salmonella (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Propionivibrio (unk species)	0.00	0.01	0.00	0.01	0.00	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00
Paracraurococcus (unk species)	0.00	0.01	0.00	0.00	0.00	0.01	0.02	0.00	0.02	0.00	0.00	0.00	0.00
Holophaga (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Escherichia coli	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Defluvibacter (unk	0.00	0.00	0.00	0.00	0.00	0.06	0.08	0.00	0.02	0.00	0.00	0.00	0.00

species)													
Brachymonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.02	0.00	0.00	0.00	0.00
Bacteriovoracaceae (unk genus)	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Spirochaeta aurantia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00
Synechococcus sp BS 5	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
Pseudomonas nitroreducens	0.00	0.00	0.00	0.00	0.00	0.25	0.08	0.02	0.00	0.00	0.00	0.00	0.00
Cyanobium sp 0BB24S01	0.13	0.04	0.08	0.07	0.03	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
Byssovorax (unk species)	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.02	0.00	0.00	0.00	0.00	0.00
Nitrospiraceae (unk genus)	0.00	0.00	0.00	0.00	0.00	1.62	2.44	0.02	0.00	0.00	0.00	0.00	0.00
Mesorhizobium (unk species)	0.00	0.00	0.00	0.00	0.00	1.04	1.49	0.02	0.00	0.00	0.00	0.00	0.00
Herbaspirillum putei	0.00	0.00	0.00	0.01	0.01	0.06	0.13	0.02	0.00	0.00	0.00	0.00	0.00
Flavobacteriaceae (unk genus)	0.01	0.00	0.03	0.00	0.01	0.02	0.04	0.02	0.00	0.00	0.00	0.00	0.00
Alvinella pompejana	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.00
TM7 uncultured	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Thermomicrobia (class) (unk order)	0.00	0.00	0.00	0.00	0.01	0.08	0.04	0.01	0.00	0.00	0.00	0.00	0.00
Sphingomonas faeni	0.00	0.00	0.03	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Samsonia erythrinae	0.00	0.00	0.00	0.00	0.00	0.15	0.20	0.01	0.00	0.00	0.00	0.00	0.00
Pseudomonas hibiscicola	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.00
Pelomonas saccharophila	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Pedomicrobium (unk species)	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Paracoccus (unk species)	0.02	0.02	0.00	0.02	0.01	0.06	0.08	0.01	0.00	0.00	0.00	0.00	0.00
Ochrobactrum (unk species)	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Massilia timonae	0.00	0.00	0.00	0.00	0.00	1.08	1.30	0.01	0.00	0.00	0.00	0.00	0.00

Lysobacter antibioticus	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Hydrogenophilales (unk family)	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00
Fucus vesiculosus(notFound)	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00
Dyella (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Dinoroseobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Coriobacteriales (unk family)	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00
Brevibacteriaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.01	0.00	0.00	0.00	0.00	0.00
Beijerinckia indica	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Auxenochlorella protothecoides(notFound)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Aminobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Acinetobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.01	0.00	0.00	0.00	0.00	0.00
Zoogloea ramigera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zoogloea oryzae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zoogloea (unk species)	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Yokenella sp	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Xylophilus ampelinus	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Xylophilus (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Xylella (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Xanthomonadaceae (unk genus)	0.02	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
WS3 uncultured	0.00	0.01	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00
WS3 (genus) (unk species)	0.02	0.05	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WS3 (family) (unk genus)	0.00	0.01	0.01	0.02	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00

WS3 (class) (unk order)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wollea saccata ACCS 045	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Verrucomicrobiae (unk order)	0.00	0.00	0.00	0.00	0.00	0.01	0.14	0.00	0.00	0.00	0.00	0.00	0.00
Verrucomicrobia (unk class)	0.00	0.00	0.00	0.00	0.00	0.27	0.26	0.00	0.00	0.00	0.00	0.00	0.00
TM7 (order) (unk family)	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
TM7 (family) (unk genus)	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.00
TM7 (class) (unk order)	0.00	0.00	0.00	0.00	0.00	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Tiedjeia sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tiedjeia (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Thermomicrobiaceae (unk genus)	0.01	0.00	0.00	0.01	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Thalassiosira pseudonana(notFound)	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Thalassiosira eccentrica(notFound)	0.05	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Syntrophorhabdus (unk species)	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Syntrophorhabdaceae (unk genus)	0.01	0.00	0.00	0.02	0.00	0.07	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Syntrophobacterales (unk family)	0.00	0.00	0.00	0.00	0.00	0.01	0.20	0.00	0.00	0.00	0.00	0.00	0.00
Synechocystis sp PCC 6803	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Synechococcus sp WH 8102	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Synechococcus sp EW15	0.02	0.02	0.00	0.01	0.03	0.03	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Synechococcus sp CC9902	0.00	0.02	0.00	0.01	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00
Streptomyces (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Stigonematales (unk	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

family)													
Stenotrophomonas rhizophila	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stenotrophomonas maltophilia	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Stenotrophomonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Staphylococcus saprophyticus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spirochaetes (unk class)	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Spirochaetes (class) (unk order)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spirillum volutans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sphingopyxis terrae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sphingopyxis chilensis	0.00	0.00	0.00	0.00	0.00	0.02	0.11	0.00	0.00	0.00	0.00	0.00	0.00
Sphingomonas azotifigens	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Sphingomonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sphingobium indicum	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sphingobium (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Spartobacteriaceae (unk family)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spartobacteria (unk order)	0.01	0.02	0.01	0.01	0.00	0.03	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Skeletonema costatum(notFound)	0.02	0.00	0.00	0.00	0.00	0.23	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Simplicispira (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Shewanellaceae (unk genus)	0.00	0.01	0.00	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Shewanella oneidensis	0.00	0.00	0.00	0.00	0.00	0.06	0.04	0.00	0.00	0.00	0.00	0.00	0.00

Shewanella (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Serratia marcescens	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Saprospiraceae (unk genus)	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Salmonella enterica	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ruegeria (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Rubrobacterales (unk family)	0.02	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Roseomonas mucosa	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rikenellaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Riemerella (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Rhodopseudomonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rhizosolenia setigera(notFound)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rhizobium leguminosarum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rhizobium etli	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rhizobiaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Pseudoxanthomonas suwonensis	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudoxanthomonas mexicana	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas trivialis	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas straminea	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas mosselii	0.00	0.00	0.00	0.00	0.00	0.19	0.11	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas lutea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas lini	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas jessenii	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas graminis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas chlororaphis	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00

Pseudomonas aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas (unk species)	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudaminobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Propionibacterium acnes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Promicromonosporaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Procabacteriales (unk family)	0.00	0.00	0.00	0.00	0.00	0.08	0.29	0.00	0.00	0.00	0.00	0.00	0.00
Prevotellaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.03	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Porphyrobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Polyangium (unk species)	0.00	0.01	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Polaromonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pleurocapsales (family) (unk genus)	0.07	0.04	0.01	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Plesiomonas shigelloides	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pleomorphomonas (unk species)	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Planktothrix (unk species)	0.00	0.01	0.00	0.00	0.00	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Planctomycetales (unk family)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phyllobacteriaceae (unk genus)	0.03	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Pelomonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pelobacter (unk species)	0.00	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Pedomicrobium australicum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Patulibacteraceae (unk	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

genus)													
Paracoccus kondratievae	0.00	0.00	0.00	0.00	0.00	0.10	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Paenibacillus amylolyticus	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Paenibacillus (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Ottowia (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OP10 (unk class)	0.00	0.00	0.00	0.00	0.00	0.07	0.04	0.00	0.00	0.00	0.00	0.00	0.00
OP10 (family) (unk genus)	0.10	0.04	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OP10 (class) (unk order)	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OD1 (order) (unk family)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OD1 (family) (unk genus)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ochrobactrum anthropi	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Oceanospirillales (unk family)	0.06	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Novosphingobium aromaticivorans	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Novosphingobium (unk species)	0.01	0.03	0.04	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nostocales (family) (unk genus)	0.00	0.00	0.01	0.01	0.00	0.52	0.97	0.00	0.00	0.00	0.00	0.00	0.00
Nosocales (unk family)	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Nocardiaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Nitrospira (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitrosospira (unk species)	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Neisseriales (unk family)	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Neisseria (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Naxibacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Nakamurellaceae (unk genus)	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Morganella morganii	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Morganella (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microcystis viridis	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Microcystis sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microcystis aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microcystaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Microbispora (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microbacterium oleivorans	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Microbacterium maritypicum	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Methylophilus methylotrophus	0.00	0.00	0.01	0.01	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Methylomonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methylocystis (unk species)	0.00	0.01	0.06	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methylococcales (unk family)	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Methylococcaceae (unk genus)	0.01	0.03	0.00	0.00	0.00	0.03	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Methylocapsa acidiphila	0.00	0.01	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Methylobacterium populi	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Methylobacterium (unk species)	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mesorhizobium amorphae	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Magnetospirillum (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Lysinibacillus sphaericus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lysinibacillus (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leptothrix mobilis	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leptothrix discophora	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Leptothrix cholodnii	0.00	0.00	0.00	0.00	0.00	0.02	0.15	0.00	0.00	0.00	0.00	0.00	0.00
Leptolinea (unk species)	0.00	0.00	0.00	0.00	0.00	0.14	0.19	0.00	0.00	0.00	0.00	0.00	0.00
Legionellales (unk family)	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Legionella lytica	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leclercia sp	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Leclercia (unk species)	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Klebsiella pneumoniae	0.00	0.00	0.01	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Klebsiella granulomatis	0.00	0.00	0.00	0.00	0.00	0.06	0.09	0.00	0.00	0.00	0.00	0.00	0.00
Klebsiella (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kitasatospora kifunensis	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hylemonella gracilis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hydrogenophilaceae (unk genus)	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hydrogenophaga defluvii	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Hydrogenophaga atypica	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Halomonadaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Haloanella (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hahella chejuensis	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hahella (unk species)	0.01	0.01	0.00	0.01	0.02	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Hafnia alvei	0.00	0.00	0.00	0.00	0.00	0.13	0.26	0.00	0.00	0.00	0.00	0.00	0.00
Haematobacter massiliensis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Haematobacter (unk species)	0.00	0.00	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Grimontella sp	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Grimontella (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gloeobacteria (unk order)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Giesbergeria sinuosa	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Giesbergeria (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Geobacteraceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00

Geobacter psychrophilus	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Geobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gemmatimonadetes (unk class)	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gemmatimonadaceae (unk genus)	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gammaproteobacteria (unk order)	0.02	0.06	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gallionellaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Fusobacteriaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Fulvimarina (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Friedmanniella (unk species)	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Flexibacteraceae (unk genus)	0.02	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Flavobacterium succinicans	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Flavobacterium limicola	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Flavobacterium johnsoniae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Firmicutes (unk class)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Exiguobacterium acetylicum	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Ewingella sp	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Ewingella (unk species)	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Escherichia (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Erysipelotrichales (unk family)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Erwinia billingiae	0.00	0.00	0.00	0.00	0.00	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Epsilonproteobacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

(unk order)													
Enterobacteriales (unk family)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Enterobacteriaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Enterobacter cloacae	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Enterobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ensifer adhaerens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Elizabethkingia (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ectothiorhodospiraceae (unk genus)	0.00	0.01	0.04	0.02	0.01	0.30	0.20	0.00	0.00	0.00	0.00	0.00	0.00
Dyella koreensis	0.00	0.00	0.00	0.00	0.00	0.07	0.15	0.00	0.00	0.00	0.00	0.00	0.00
Desulforegula conservatrix	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Desulfobacteraceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Delftia acidovorans	0.00	0.00	0.00	0.00	0.00	0.10	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Delftia (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dechloromonas aromatica	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dechloromonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.17	0.00	0.00	0.00	0.00	0.00	0.00
Cystobacteraceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.04	0.07	0.00	0.00	0.00	0.00	0.00	0.00
Cylindrospermopsis raciborskii BM	0.00	0.00	0.00	0.00	0.00	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Cyanobium sp Y0011	0.05	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Cyanobacteria (family) (unk genus)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Curvibacter delicatus	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Cupriavidus taiwanensis	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00

Cupriavidus necator	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cryocola sp	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Crocinitomix sp	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crocebacterium (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crenothrix (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Comamonas testosteroni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Comamonas terrigena	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Clostridiales incertae sedis (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Citrobacter murliniae	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Citreicella (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chryseobacterium indologenes	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chryseobacterium hispanicum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chryseobacterium daecheongense	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Chryseobacterium (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Chroococcales uncultured	0.01	0.02	0.01	0.00	0.01	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Chroococcales (genus) (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Chromobacterium violaceum	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Chromobacterium (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Chlorophyta uncultured(notFound)	0.07	0.02	0.03	0.01	0.01	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Chlorophyta uncultured	0.05	0.00	0.00	0.00	0.00	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Chloroflexi (unk class)	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Chloroflexales (unk	0.00	0.00	0.00	0.00	0.00	0.11	0.10	0.00	0.00	0.00	0.00	0.00	0.00

family)													
Chloroflexaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Chlorella ellipsoidea(notFound)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Chitinimonas taiwanensis	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Chitinimonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.06	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Chitinibacter tainanensis	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chitinibacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cellvibrio mixtus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cellvibrio fulvus	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Cellulomonadaceae (unk genus)	0.01	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Celerinatantimonadaceae (unk genus)	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caulobacteraceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Candidatus Reyranella sp	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Candidatus Planktoluna sp	0.06	0.03	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Candidatus Monilibacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Candidatus Azospirillum sp	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Candidatus Aquiluna (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Campylobacteraceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Caldilineae (unk order)	0.02	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caldilinea sp	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caldilinea (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Byssovorax sp	0.00	0.00	0.00	0.00	0.00	0.06	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Buttiauxella brennerae	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Burkholderia phytofirmans	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Burkholderia glathei	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brevundimonas subvibrioides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brevundimonas nasdae	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Brevundimonas diminuta	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Brevundimonas (unk species)	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brenneria (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Bosea vestrisii	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bordetella avium	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bdellovibrionales (unk family)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Bartonella henselae(notFound)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Bacteroides intestinalis	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Bacteroides (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteria incertae sedis (unk class)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus subtilis	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus pumilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus cereus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacilli (unk order)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillales (unk family)	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillaceae (unk genus)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Azospirillum oryzae	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Azospirillum lipoferum	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Azospirillum (unk	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00

species)													
Azoarcus evansii	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Azoarcus (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Auxenochlorella protothecoides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Asticcacaulis excentricus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arthrobacter nicotinovorans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arcobacter cryaerophilus	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Arcobacter butzleri	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aranicola sp	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Aranicola (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Aquitalea magnusonii	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Anaeromyxobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aminomonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Alteromonadales (unk family)	0.01	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Alteromonadaceae (unk genus)	0.06	0.06	0.06	0.06	0.00	0.01	0.32	0.00	0.00	0.00	0.00	0.00	0.00
Alishewanella fetalis	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alicycliphilus denitrificans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alcaligenaceae (unk genus)	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Afipia broomeae	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Afipia (unk species)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonas veronii	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonas schubertii	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonas salmonicida	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonas molluscorum	0.00	0.00	0.00	0.00	0.00	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonas media	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Aeromonas jandaei	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonas hydrophila	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonas eucrenophila	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonas allosaccharophila	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonas (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Aeromonadales (unk family)	0.01	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00
Actinoplanes teichomyceticus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acinetobacter calcoaceticus	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Acinetobacter baumannii	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Acidovorax delafieldii	0.00	0.00	0.00	0.00	0.00	0.02	0.08	0.00	0.00	0.00	0.00	0.00	0.00
Acidovorax defluvii	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Acidovorax (unk species)	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Acidobacteriales (unk family)	0.00	0.00	0.00	0.00	0.00	0.06	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Acidobacteria (class) (unk order)	0.00	0.00	0.00	0.00	0.00	0.04	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Acidisphaera rubrifaciens	0.00	0.00	0.00	0.00	0.01	0.06	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Acetobacter (unk species)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00

Species Percentages for Ohio River and Guyandotte Sampling Sites. OH=Ohio bank, CH= Channel, WV= West Virginia bank, GUY= Guyandotte River, CH GUY= Ohio River Channel at the mouth of the Guyandotte, OH GUY= Ohio bank across from mouth of the Guyandotte, and DS GUY= Ohio River downstream from Guyandotte. Number after each site refers to sampling bottle.

Table 4.

Name	CH 3C	CH 4C	CH 5C
Aeromonas veronii	30.14	14.08	19.39
Aeromonas schubertii	16.18	5.75	5.35
Aeromonas jandaei	5.68	3.40	3.59
Vogesella (unk species)	4.92	14.01	10.78
Alishewanella (unk species)	4.67	15.70	10.05
Bacillus thuringiensis	3.32	2.90	10.30
Enterobacter cloacae	3.31	3.14	2.34
Alteromonadaceae (unk genus)	2.16	8.98	5.33
Flavobacteriaceae (unk genus)	1.79	0.95	0.57
Pseudomonas putida	1.68	0.99	0.65
Neisseriaceae (unk genus)	1.52	3.45	2.68
Mitsuaria chitosanitabida	1.44	0.31	0.33
Vogesella indigofera	0.93	0.86	1.09
Comamonadaceae (unk genus)	0.88	0.71	0.93
Bacillus mycoides	0.78	2.11	2.39
Leclercia sp	0.78	1.08	0.64
Samsonia erythrinae	0.67	0.68	0.52
Noctuoidea (unk genus)	0.67	0.57	0.66
Aeromonas punctata	0.65	2.11	0.47
Burkholderiaceae (unk genus)	0.65	0.61	0.79
Escherichia coli	0.65	0.22	0.34
Pseudomonas (unk species)	0.62	0.17	0.78
Oxalobacteraceae (unk genus)	0.57	0.52	0.68
Pseudomonas straminea	0.56	0.15	0.14

Yokenella sp	0.54	0.62	0.48
Aeromonas hydrophila	0.45	1.47	0.40
Hafnia alvei	0.45	0.01	0.10
Bacillus weihenstephanensis	0.43	0.06	2.30
Roseateles (unk species)	0.43	0.03	0.02
Burkholderiales (unk family)	0.40	0.32	0.23
Shewanella (unk species)	0.34	0.04	0.09
Pseudomonas mendocina	0.34	0.03	0.07
Pseudomonas graminis	0.33	0.32	0.02
Shewanella putrefaciens	0.33	0.01	0.02
Gammaproteobacteria (unk order)	0.31	1.13	0.91
Aeromonas (unk species)	0.29	0.41	0.23
Enterobacteriaceae (unk genus)	0.29	0.34	0.24
Pseudomonas stutzeri	0.29	0.27	0.09
Bacillus cereus	0.29	0.09	1.07
Alteromonadales (unk family)	0.28	0.96	0.58
Bacteria (unk phylum)	0.28	0.52	0.41
Pseudomonadaceae (unk genus)	0.28	0.08	0.47
Stenotrophomonas maltophilia	0.26	0.14	0.15
Chitinibacter tainanensis	0.25	0.99	0.10
Rhodocyclaceae (unk genus)	0.25	0.31	0.41
Pelomonas (unk species)	0.23	0.08	0.08
Aeromonadaceae (unk genus)	0.22	0.36	0.28
Pseudomonas argentinensis	0.22	0.01	0.06
Chitinimonas taiwanensis	0.22	0.00	0.00
Chromobacterium violaceum	0.20	0.08	0.02

Acidovorax delafieldii	0.20	0.00	0.02
Alishewanella fetalis	0.19	0.80	0.46
Aranicola sp	0.19	0.21	0.06
Ewingella sp	0.19	0.17	0.26
Elizabethkingia (unk species)	0.17	0.06	0.04
Ochrobactrum anthropi	0.17	0.00	0.05
Tiedjeia sp	0.16	0.15	0.05
Enterobacteriales (unk family)	0.16	0.14	0.07
Pseudomonas fluorescens	0.16	0.06	0.05
Stenotrophomonas rhizophila	0.16	0.00	0.00
Chromatiales (unk family)	0.14	0.50	0.33
Flavobacterium (unk species)	0.14	0.13	0.05
Samsonia (unk species)	0.14	0.12	0.15
Candidatus Rhizobium sp	0.14	0.05	0.02
Shewanellaceae (unk genus)	0.14	0.02	0.02
Chryseobacterium (unk species)	0.12	0.06	0.05
Ewingella (unk species)	0.12	0.05	0.04
Proteobacteria (unk class)	0.11	0.23	0.19
Hydrogenophaga atypica	0.11	0.12	0.22
Enterobacter (unk species)	0.11	0.12	0.06
Bacillales (unk family)	0.11	0.10	0.11
Bacillaceae (unk genus)	0.11	0.09	0.26
Chitinibacter (unk species)	0.11	0.05	0.11
Xylella (unk species)	0.11	0.02	0.00
Plesiomonas shigelloides	0.11	0.01	0.00
Methylibium (unk species)	0.11	0.00	0.01
Betaproteobacteria (unk	0.09	0.22	0.23

order)			
Curvibacter (unk species)	0.09	0.08	0.48
Stenotrophomonas (unk species)	0.09	0.08	0.02
Acidovorax defluvii	0.09	0.02	0.10
Mitsuaria (unk species)	0.09	0.02	0.02
Pseudomonas mosselii	0.09	0.01	0.02
Burkholderia ambifaria	0.08	0.06	0.02
Xanthomonadaceae (unk genus)	0.08	0.05	0.07
Herbaspirillum seropedicae	0.08	0.04	0.03
Neisseriales (unk family)	0.06	0.25	0.22
Morganella morganii	0.06	0.06	0.02
Curvibacter gracilis	0.06	0.05	0.20
Bacillus pumilus	0.06	0.04	0.05
Rhizobium (unk species)	0.06	0.03	0.02
Herbaspirillum (unk species)	0.06	0.03	0.02
Novosphingobium aromaticivorans	0.06	0.02	0.02
Chromobacterium (unk species)	0.05	0.19	0.01
Bacillus (unk species)	0.05	0.13	1.64
Aeromonas salmonicida	0.05	0.12	0.04
Comamonas testosteroni	0.05	0.09	0.00
Tiedjeia (unk species)	0.05	0.07	0.01
Aeromonadales (unk family)	0.05	0.04	0.05
Bacillus licheniformis	0.05	0.02	0.00
Salmonella enterica	0.05	0.01	0.01
Hydrogenophaga (unk species)	0.05	0.00	0.02
Shewanella oneidensis	0.05	0.00	0.02
Comamonas terrigena	0.05	0.00	0.00

Asticcacaulis excentricus	0.05	0.00	0.00
Serratia marcescens	0.03	0.50	0.32
Flavobacteriales (unk family)	0.03	0.06	0.07
Pseudomonas luteola	0.03	0.05	0.02
Malikia (unk species)	0.03	0.04	0.02
Alcaligenes faecalis	0.03	0.04	0.01
Delftia acidovorans	0.03	0.02	0.16
Herbaspirillum lusitanum	0.03	0.01	0.03
Pelomonas saccharophila	0.03	0.01	0.02
Cupriavidus necator	0.03	0.01	0.00
Massilia timonae	0.03	0.00	0.08
Serratia proteamaculans	0.03	0.00	0.03
Shewanella pealeana	0.03	0.00	0.00
Erwinia billingiae	0.03	0.00	0.00
Aquitalea magnusonii	0.03	0.00	0.00
Azospirillum (unk species)	0.02	0.09	0.00
Bacilli (unk order)	0.02	0.07	0.08
Pseudoxanthomonas mexicana	0.02	0.05	0.03
Xylophilus ampelinus	0.02	0.03	0.05
Rhodocyclales (unk family)	0.02	0.03	0.03
Cupriavidus (unk species)	0.02	0.03	0.02
Firmicutes (unk class)	0.02	0.03	0.01
Pseudomonas jessenii	0.02	0.02	0.02
Dechloromonas aromatica	0.02	0.02	0.01
Herbaspirillum chlorophenolicum	0.02	0.02	0.02
Xanthomonadales (unk family)	0.02	0.02	0.01
Chryseobacterium indologenes	0.02	0.02	0.00
Acinetobacter (unk species)	0.02	0.01	0.12

Flavobacteria (unk order)	0.02	0.01	0.04
Microbacteriaceae (unk genus)	0.02	0.01	0.03
Comamonas (unk species)	0.02	0.01	0.01
Pseudoxanthomonas suwonensis	0.02	0.01	0.00
Pseudomonas hibiscicola	0.02	0.01	0.00
Acidovorax (unk species)	0.02	0.01	0.00
Moraxellaceae (unk genus)	0.02	0.00	0.22
Pseudomonas nitroreducens	0.02	0.00	0.18
Sphingobium (unk species)	0.02	0.00	0.06
Sphingobacteriaceae (unk genus)	0.02	0.00	0.03
Acidovorax avenae	0.02	0.00	0.03
Ramlibacter (unk species)	0.02	0.00	0.02
Sphingobacteriales (unk family)	0.02	0.00	0.01
Ensifer adhaerens	0.02	0.00	0.01
Yersinia (unk species)	0.02	0.00	0.00
Syntrophorhabdus (unk species)	0.02	0.00	0.00
Salmonella (unk species)	0.02	0.00	0.00
Rhizobium leguminosarum	0.02	0.00	0.00
Rhizobium etli	0.02	0.00	0.00
Pseudomonas lini	0.02	0.00	0.00
Pseudomonas geniculata	0.02	0.00	0.00
Pseudomonas fulva	0.02	0.00	0.00
Phyllobacteriaceae (unk genus)	0.02	0.00	0.00
Ochrobactrum (unk species)	0.02	0.00	0.00
Microbacterium thalassium	0.02	0.00	0.00
Microbacterium (unk	0.02	0.00	0.00

species)			
Hydrogenophaga taeniospiralis	0.02	0.00	0.00
Fulvimarina (unk species)	0.02	0.00	0.00
Defluvibacter (unk species)	0.02	0.00	0.00
Burkholderia phytofirmans	0.02	0.00	0.00
Brevundimonas subvibrioides	0.02	0.00	0.00
Citrobacter murliniae	0.00	0.21	0.02
Zoogloea (unk species)	0.00	0.12	0.00
Klebsiella pneumoniae	0.00	0.12	0.00
Staphylococcus saprophyticus	0.00	0.11	0.00
Paenibacillus amylolyticus	0.00	0.08	0.04
Candidatus Azospirillum sp	0.00	0.08	0.00
Massilia (unk species)	0.00	0.05	0.09
Malikia granosa	0.00	0.05	0.00
Herbaspirillum putei	0.00	0.05	0.00
Rhodospirillaceae (unk genus)	0.00	0.05	0.00
Acidovorax temperans	0.00	0.04	0.16
Pseudomonadales (unk family)	0.00	0.03	0.04
Escherichia (unk species)	0.00	0.03	0.03
Lysinibacillus sphaericus	0.00	0.03	0.02
Aquitalea (unk species)	0.00	0.03	0.00
Sphingomonadaceae (unk genus)	0.00	0.02	0.05
Acinetobacter baumannii	0.00	0.02	0.02
Sphingopyxis chilensis	0.00	0.02	0.01
Novosphingobium (unk species)	0.00	0.02	0.01

Grimontella (unk species)	0.00	0.02	0.01
Rhizobiaceae (unk genus)	0.00	0.02	0.00
Magnetospirillum magneticum	0.00	0.02	0.00
Caulobacteraceae (unk genus)	0.00	0.02	0.00
Alphaproteobacteria (unk order)	0.00	0.02	0.00
Achromobacter xylosoxidans	0.00	0.02	0.06
Chromatiaceae (unk genus)	0.00	0.02	0.02
Serratia (unk species)	0.00	0.02	0.01
Rubrivivax (unk species)	0.00	0.02	0.01
Polynucleobacter (unk species)	0.00	0.02	0.01
Brevundimonas (unk species)	0.00	0.02	0.01
Alcaligenaceae (unk genus)	0.00	0.02	0.01
Methylophilus methylotrophus	0.00	0.02	0.00
Chryseobacterium gleum	0.00	0.02	0.00
Aeromonas media	0.00	0.02	0.00
Pseudomonas chlororaphis	0.00	0.01	0.09
Bosea vestrisii	0.00	0.01	0.06
Paenibacillus (unk species)	0.00	0.01	0.02
Paenibacillaceae (unk genus)	0.00	0.01	0.02
Pseudomonas lutea	0.00	0.01	0.01
Micrococcaceae (unk genus)	0.00	0.01	0.01
Haematobacter (unk species)	0.00	0.01	0.01
Giesbergeria (unk species)	0.00	0.01	0.01
Flavobacterium johnsoniae	0.00	0.01	0.01
Bacteroidetes (unk class)	0.00	0.01	0.01
Aeromonas molluscorum	0.00	0.01	0.01

Sphingomonas azotifigens	0.00	0.01	0.00
Riemerella (unk species)	0.00	0.01	0.00
Rhodospirillales (unk family)	0.00	0.01	0.00
Rhodoferax (unk species)	0.00	0.01	0.00
Paenibacillus chitinolyticus	0.00	0.01	0.00
Nocardiaceae (unk genus)	0.00	0.01	0.00
Morganella (unk species)	0.00	0.01	0.00
Lysinibacillus (unk species)	0.00	0.01	0.00
Leclercia (unk species)	0.00	0.01	0.00
Klebsiella granulomatis	0.00	0.01	0.00
Hylemonella gracilis	0.00	0.01	0.00
Hydrogenophaga defluvii	0.00	0.01	0.00
Dechloromonas (unk species)	0.00	0.01	0.00
Chryseobacterium taiwanense	0.00	0.01	0.00
Chryseobacterium daecheongense	0.00	0.01	0.00
Candidatus Cuticobacterium sp	0.00	0.01	0.00
Arthrobacter nicotinovorans	0.00	0.01	0.00
Aranicola (unk species)	0.00	0.01	0.00
Alcaligenes (unk species)	0.00	0.01	0.00
Pseudomonas otitidis	0.00	0.00	0.42
Pseudomonas aeruginosa	0.00	0.00	0.34
Hydrogenophilaceae (unk genus)	0.00	0.00	0.15
Actinomycetales (unk family)	0.00	0.00	0.07
Delftia (unk species)	0.00	0.00	0.05
Sphingobacteria (unk order)	0.00	0.00	0.04
Alvinella pompejana	0.00	0.00	0.04

Streptomycetaceae (unk genus)	0.00	0.00	0.03
Actinobacteria (class) (unk order)	0.00	0.00	0.03
Leptothrix (unk species)	0.00	0.00	0.02
Giesbergeria sinuosa	0.00	0.00	0.02
Curvibacter delicatus	0.00	0.00	0.02
Xylophilus (unk species)	0.00	0.00	0.02
Sphingomonas (unk species)	0.00	0.00	0.02
Sanguibacteraceae (unk genus)	0.00	0.00	0.02
Rhodobaca (unk species)	0.00	0.00	0.02
Paracoccus (unk species)	0.00	0.00	0.02
Methylocapsa acidiphila	0.00	0.00	0.02
Methylocapsa (unk species)	0.00	0.00	0.02
Lysobacter antibioticus	0.00	0.00	0.02
Halomonadaceae (unk genus)	0.00	0.00	0.02
Haloanella (unk species)	0.00	0.00	0.02
Candidatus Planktoluna sp	0.00	0.00	0.02
Beijerinckia (unk species)	0.00	0.00	0.02
Bacillus subtilis	0.00	0.00	0.02
Synechococcus uncultured	0.00	0.00	0.01
Saprospiraceae (unk genus)	0.00	0.00	0.01
Rhodoferax ferrireducens	0.00	0.00	0.01
Rhodobacteraceae (unk genus)	0.00	0.00	0.01
Rhodobacter (unk species)	0.00	0.00	0.01
Prevotellaceae (unk genus)	0.00	0.00	0.01
Polynucleobacter necessarius	0.00	0.00	0.01
Polyangiaceae (unk genus)	0.00	0.00	0.01

Paracraurococcus (unk species)	0.00	0.00	0.01
Opitutaceae (unk genus)	0.00	0.00	0.01
OP10 unclassified	0.00	0.00	0.01
Neisseria (unk species)	0.00	0.00	0.01
Microbacterium oleivorans	0.00	0.00	0.01
Methylophilales (unk family)	0.00	0.00	0.01
Methylophilaceae (unk genus)	0.00	0.00	0.01
Magnetospirillum (unk species)	0.00	0.00	0.01
Lysobacter (unk species)	0.00	0.00	0.01
Legionellaceae (unk genus)	0.00	0.00	0.01
Kineosporiaceae (unk genus)	0.00	0.00	0.01
Hyphomicrobiaceae (unk genus)	0.00	0.00	0.01
Hydrogenophilales (unk family)	0.00	0.00	0.01
Grimontella sp	0.00	0.00	0.01
Geobacteraceae (unk genus)	0.00	0.00	0.01
Dyella (unk species)	0.00	0.00	0.01
Dermabacteraceae (unk genus)	0.00	0.00	0.01
Candidatus Reyranella sp	0.00	0.00	0.01
Buttiauxella brennerae	0.00	0.00	0.01
Burkholderia cenocepacia	0.00	0.00	0.01
Burkholderia (unk species)	0.00	0.00	0.01
Brevibacterium (unk species)	0.00	0.00	0.01
Brenneria (unk species)	0.00	0.00	0.01
Bradyrhizobiaceae (unk genus)	0.00	0.00	0.01

Actinobacteria (unk class)	0.00	0.00	0.01
Acinetobacter calcoaceticus	0.00	0.00	0.01
Zoogloea ramigera	0.00	0.00	0.00
Zoogloea oryzae	0.00	0.00	0.00
Xanthobacteraceae (unk genus)	0.00	0.00	0.00
WS3 uncultured	0.00	0.00	0.00
WS3 (genus) (unk species)	0.00	0.00	0.00
WS3 (family) (unk genus)	0.00	0.00	0.00
WS3 (class) (unk order)	0.00	0.00	0.00
Wollea saccata ACCS 045	0.00	0.00	0.00
Verrucomicrobiales (unk family)	0.00	0.00	0.00
Verrucomicrobiae (unk order)	0.00	0.00	0.00
Verrucomicrobiaceae (unk genus)	0.00	0.00	0.00
Verrucomicrobia (unk class)	0.00	0.00	0.00
Variovorax (unk species)	0.00	0.00	0.00
TM7 uncultured	0.00	0.00	0.00
TM7 (unk class)	0.00	0.00	0.00
TM7 (order) (unk family)	0.00	0.00	0.00
TM7 (genus) (unk species)	0.00	0.00	0.00
TM7 (family) (unk genus)	0.00	0.00	0.00
TM7 (class) (unk order)	0.00	0.00	0.00
Thiotrichales (unk family)	0.00	0.00	0.00
Thermomicrobiales (unk family)	0.00	0.00	0.00
Thermomicrobiaceae (unk genus)	0.00	0.00	0.00
Thermomicrobia (class) (unk order)	0.00	0.00	0.00

Thauera (unk species)	0.00	0.00	0.00
Thalassiosira pseudonana(notFound)	0.00	0.00	0.00
Thalassiosira pseudonana	0.00	0.00	0.00
Thalassiosira eccentrica(notFound)	0.00	0.00	0.00
Thalassiosira eccentrica	0.00	0.00	0.00
Syntrophorhabdaceae (unk genus)	0.00	0.00	0.00
Syntrophobacterales (unk family)	0.00	0.00	0.00
Synechocystis sp PCC 6803	0.00	0.00	0.00
Synechococcus sp WH 8102	0.00	0.00	0.00
Synechococcus sp WH 8101	0.00	0.00	0.00
Synechococcus sp WH 7803	0.00	0.00	0.00
Synechococcus sp LBG2	0.00	0.00	0.00
Synechococcus sp LBB3	0.00	0.00	0.00
Synechococcus sp EW15	0.00	0.00	0.00
Synechococcus sp CCMP839	0.00	0.00	0.00
Synechococcus sp CC9902	0.00	0.00	0.00
Synechococcus sp CC9311	0.00	0.00	0.00
Synechococcus sp BS 5	0.00	0.00	0.00
Synechococcus sp 0BB26S03	0.00	0.00	0.00
Synechococcus sp 0BB22S0	0.00	0.00	0.00
Synechococcus (unk species)	0.00	0.00	0.00
Streptomyces sp	0.00	0.00	0.00
Streptomyces lavendulae	0.00	0.00	0.00
Streptomyces (unk species)	0.00	0.00	0.00
Stigonematales (unk family)	0.00	0.00	0.00
Sterolibacterium (unk species)	0.00	0.00	0.00
Spirulina (unk species)	0.00	0.00	0.00

Spirochaetes (unk class)	0.00	0.00	0.00
Spirochaetes (class) (unk order)	0.00	0.00	0.00
Spirochaetales (unk family)	0.00	0.00	0.00
Spirochaetaceae (unk genus)	0.00	0.00	0.00
Spirochaeta aurantia	0.00	0.00	0.00
Spirillum volutans	0.00	0.00	0.00
Sphingopyxis witflariensis	0.00	0.00	0.00
Sphingopyxis terrae	0.00	0.00	0.00
Sphingopyxis alaskensis	0.00	0.00	0.00
Sphingopyxis (unk species)	0.00	0.00	0.00
Sphingomonas phyllosphaerae	0.00	0.00	0.00
Sphingomonas faeni	0.00	0.00	0.00
Sphingomonadales (unk family)	0.00	0.00	0.00
Sphingobium indicum	0.00	0.00	0.00
Sphingobium herbicidovorans	0.00	0.00	0.00
Spartobacteriaceae (unk family)	0.00	0.00	0.00
Spartobacteria (unk order)	0.00	0.00	0.00
Solibacteres (unk order)	0.00	0.00	0.00
Solibacterales (unk family)	0.00	0.00	0.00
Solibacteraceae (unk genus)	0.00	0.00	0.00
Skeletonema costatum(notFound)	0.00	0.00	0.00
Skeletonema costatum	0.00	0.00	0.00
Simplicispira (unk species)	0.00	0.00	0.00
Shinella (unk species)	0.00	0.00	0.00
Schlegelella thermodepolymerans	0.00	0.00	0.00

Schlegelella (unk species)	0.00	0.00	0.00
Scherffelia dubia(notFound)	0.00	0.00	0.00
Sanguibacter (unk species)	0.00	0.00	0.00
Ruegeria (unk species)	0.00	0.00	0.00
Rubrobacterales (unk family)	0.00	0.00	0.00
Roseomonas mucosa	0.00	0.00	0.00
Roseomonas (unk species)	0.00	0.00	0.00
Rikenellaceae (unk genus)	0.00	0.00	0.00
Rickettsiales (unk family)	0.00	0.00	0.00
Rickettsiaceae (unk genus)	0.00	0.00	0.00
Rickettsia (unk species)	0.00	0.00	0.00
Rhodopseudomonas palustris	0.00	0.00	0.00
Rhodopseudomonas (unk species)	0.00	0.00	0.00
Rhodomicrobium sp	0.00	0.00	0.00
Rhodobacterales (unk family)	0.00	0.00	0.00
Rhodobacter sphaeroides	0.00	0.00	0.00
Rhizosolenia setigera(notFound)	0.00	0.00	0.00
Rhizobium giardinii	0.00	0.00	0.00
Rhizobiales (unk family)	0.00	0.00	0.00
Raphidiopsis curvata HB1	0.00	0.00	0.00
Ramlibacter tataouinensis	0.00	0.00	0.00
Pseudoxanthomonas (unk species)	0.00	0.00	0.00
Pseudomonas trivialis	0.00	0.00	0.00
Pseudaminobacter (unk species)	0.00	0.00	0.00
Prosthecobacter (unk	0.00	0.00	0.00

species)			
Propionivibrio limicola	0.00	0.00	0.00
Propionivibrio (unk species)	0.00	0.00	0.00
Propionibacterium acnes	0.00	0.00	0.00
Promicromonosporaceae (unk genus)	0.00	0.00	0.00
Procabacteriales (unk family)	0.00	0.00	0.00
Porphyrobacter (unk species)	0.00	0.00	0.00
Polyangium (unk species)	0.00	0.00	0.00
Polaromonas (unk species)	0.00	0.00	0.00
Pleurocapsales (unk family)	0.00	0.00	0.00
Pleurocapsales (family) (unk genus)	0.00	0.00	0.00
Pleomorphomonas (unk species)	0.00	0.00	0.00
Planktothrix (unk species)	0.00	0.00	0.00
Planctomycetales (unk family)	0.00	0.00	0.00
Phyllobacterium trifolii	0.00	0.00	0.00
Phyllobacterium (unk species)	0.00	0.00	0.00
Phoridium (unk species)	0.00	0.00	0.00
Phenylobacterium immobile	0.00	0.00	0.00
Phenylobacterium (unk species)	0.00	0.00	0.00
Pelobacteraceae (unk genus)	0.00	0.00	0.00
Pelobacter propionicus	0.00	0.00	0.00
Pelobacter (unk species)	0.00	0.00	0.00
Pedomicrobium australicum	0.00	0.00	0.00
Pedomicrobium (unk	0.00	0.00	0.00

species)			
Patulibacteraceae (unk genus)	0.00	0.00	0.00
Paracoccus kondratievae	0.00	0.00	0.00
Paracoccus carotinifaciens	0.00	0.00	0.00
Ottowia (unk species)	0.00	0.00	0.00
Oscillatoriales (unk family)	0.00	0.00	0.00
Oscillatoriales (family) (unk genus)	0.00	0.00	0.00
Oscillatoriaceae (unk genus)	0.00	0.00	0.00
Opitutus (unk species)	0.00	0.00	0.00
Opitutales (unk family)	0.00	0.00	0.00
Opitutae (unk order)	0.00	0.00	0.00
OP10 (unk class)	0.00	0.00	0.00
OP10 (order) (unk family)	0.00	0.00	0.00
OP10 (genus) (unk species)	0.00	0.00	0.00
OP10 (family) (unk genus)	0.00	0.00	0.00
OP10 (class) (unk order)	0.00	0.00	0.00
OD1 (unk class)	0.00	0.00	0.00
OD1 (order) (unk family)	0.00	0.00	0.00
OD1 (family) (unk genus)	0.00	0.00	0.00
OD1 (class) (unk order)	0.00	0.00	0.00
Oceanospirillales (unk family)	0.00	0.00	0.00
Oceanospirillaceae (unk genus)	0.00	0.00	0.00
Novosphingobium stygium	0.00	0.00	0.00
Novosphingobium pentaromativorans	0.00	0.00	0.00
Novosphingobium hassiacum	0.00	0.00	0.00
Nostocales (unk family)	0.00	0.00	0.00

Nostocales (family) (unk genus)	0.00	0.00	0.00
Nostocaceae (unk genus)	0.00	0.00	0.00
Nosocales (unk family)	0.00	0.00	0.00
Nitrospiraceae (unk genus)	0.00	0.00	0.00
Nitrospira moscoviensis	0.00	0.00	0.00
Nitrospira (unk species)	0.00	0.00	0.00
Nitrosospira (unk species)	0.00	0.00	0.00
Nitrosomonadales (unk family)	0.00	0.00	0.00
Nitrosomonadaceae (unk genus)	0.00	0.00	0.00
Nephroselmis olivacea(notFound)	0.00	0.00	0.00
Naxibacter (unk species)	0.00	0.00	0.00
Nakamurellaceae (unk genus)	0.00	0.00	0.00
Myxococcales (unk family)	0.00	0.00	0.00
Myxococcaceae (unk genus)	0.00	0.00	0.00
Mycobacterium pyrenivorans	0.00	0.00	0.00
Mycobacterium conceptionense	0.00	0.00	0.00
Mycobacterium (unk species)	0.00	0.00	0.00
Micromonosporaceae (unk genus)	0.00	0.00	0.00
Micromonospora matsumotoense	0.00	0.00	0.00
Microcystis wesenbergii	0.00	0.00	0.00
Microcystis viridis	0.00	0.00	0.00
Microcystis sp	0.00	0.00	0.00

Microcystis holsatica	0.00	0.00	0.00
Microcystis aeruginosa NIES-843	0.00	0.00	0.00
Microcystis aeruginosa NIES-298	0.00	0.00	0.00
Microcystis aeruginosa	0.00	0.00	0.00
Microcystaceae (unk genus)	0.00	0.00	0.00
Microbispora (unk species)	0.00	0.00	0.00
Microbacterium maritypicum	0.00	0.00	0.00
Methylovorus sp	0.00	0.00	0.00
Methylovorus (unk species)	0.00	0.00	0.00
Methylosarcina lacus	0.00	0.00	0.00
Methylosarcina (unk species)	0.00	0.00	0.00
Methylomonas (unk species)	0.00	0.00	0.00
Methylocystis (unk species)	0.00	0.00	0.00
Methylocystaceae (unk genus)	0.00	0.00	0.00
Methylococcales (unk family)	0.00	0.00	0.00
Methylococcaceae (unk genus)	0.00	0.00	0.00
Methylocaldum (unk species)	0.00	0.00	0.00
Methylobacterium radiotolerans	0.00	0.00	0.00
Methylobacterium populi	0.00	0.00	0.00
Methylobacterium (unk species)	0.00	0.00	0.00
Methylobacteriaceae (unk genus)	0.00	0.00	0.00

Methylobacillus (unk species)	0.00	0.00	0.00
Mesorhizobium ciceri	0.00	0.00	0.00
Mesorhizobium amorphae	0.00	0.00	0.00
Mesorhizobium (unk species)	0.00	0.00	0.00
Merismopedia tenuissima 0BB46S01	0.00	0.00	0.00
Merismopedia (unk species)	0.00	0.00	0.00
Magnetobacterium sp	0.00	0.00	0.00
Levilinea sp	0.00	0.00	0.00
Levilinea (unk species)	0.00	0.00	0.00
Leptothrix mobilis	0.00	0.00	0.00
Leptothrix discophora	0.00	0.00	0.00
Leptothrix cholodnii	0.00	0.00	0.00
Leptolinea (unk species)	0.00	0.00	0.00
Legionellales (unk family)	0.00	0.00	0.00
Legionella pneumophila	0.00	0.00	0.00
Legionella lytica	0.00	0.00	0.00
Legionella (unk species)	0.00	0.00	0.00
Ktedonobacteria (unk order)	0.00	0.00	0.00
Ktedonobacterales (unk family)	0.00	0.00	0.00
Ktedonobacteraceae (unk genus)	0.00	0.00	0.00
Krasilnikovia sp	0.00	0.00	0.00
Klebsiella (unk species)	0.00	0.00	0.00
Kitasatospora kifunensis	0.00	0.00	0.00
Jannaschia (unk species)	0.00	0.00	0.00
Intrasporangiaceae (unk genus)	0.00	0.00	0.00
Hyphomicrobium (unk	0.00	0.00	0.00

species)			
Hydrogenophaga pseudoflava	0.00	0.00	0.00
Holophaga (unk species)	0.00	0.00	0.00
Heterosigma akashiwo(notFound)	0.00	0.00	0.00
Helicobacteraceae (unk genus)	0.00	0.00	0.00
Haliscomenobacter (unk species)	0.00	0.00	0.00
Hahellaceae (unk genus)	0.00	0.00	0.00
Hahella chejuensis	0.00	0.00	0.00
Hahella (unk species)	0.00	0.00	0.00
Haematobacter massiliensis	0.00	0.00	0.00
Gloeobacteria (unk order)	0.00	0.00	0.00
Geobacter psychrophilus	0.00	0.00	0.00
Geobacter bemidjiensis	0.00	0.00	0.00
Geobacter (unk species)	0.00	0.00	0.00
Gemmatimonadetes (unk class)	0.00	0.00	0.00
Gemmatimonadetes (class) (unk order)	0.00	0.00	0.00
Gemmatimonadales (unk family)	0.00	0.00	0.00
Gemmatimonadaceae (unk genus)	0.00	0.00	0.00
Gallionellaceae (unk genus)	0.00	0.00	0.00
Fusobacteriaceae (unk genus)	0.00	0.00	0.00
Fucus vesiculosus(notFound)	0.00	0.00	0.00
Friedmanniella (unk species)	0.00	0.00	0.00

Fluviicola (unk species)	0.00	0.00	0.00
Flexibacteraceae (unk genus)	0.00	0.00	0.00
Flavobacterium succinicans	0.00	0.00	0.00
Flavobacterium limicola	0.00	0.00	0.00
Exiguobacterium acetylicum	0.00	0.00	0.00
Erythrobacteraceae (unk genus)	0.00	0.00	0.00
Erysipelotrichales (unk family)	0.00	0.00	0.00
Epsilonproteobacteria (unk order)	0.00	0.00	0.00
Ensifer (unk species)	0.00	0.00	0.00
Ectothiorhodospiraceae (unk genus)	0.00	0.00	0.00
Dyella koreensis	0.00	0.00	0.00
Dinoroseobacter (unk species)	0.00	0.00	0.00
Desulfuromonadales (unk family)	0.00	0.00	0.00
Desulfovibrio magneticus	0.00	0.00	0.00
Desulforegula conservatrix	0.00	0.00	0.00
Desulfobacteraceae (unk genus)	0.00	0.00	0.00
Deltaproteobacteria (unk order)	0.00	0.00	0.00
Dechloromonas hortensis	0.00	0.00	0.00
Cystobacteraceae (unk genus)	0.00	0.00	0.00
Cylindrospermopsis raciborskii BM	0.00	0.00	0.00
Cyanobium sp Y0011	0.00	0.00	0.00

Cyanobium sp LB03	0.00	0.00	0.00
Cyanobium sp 0BB42S04	0.00	0.00	0.00
Cyanobium sp 0BB24S01	0.00	0.00	0.00
Cyanobium (unk species)	0.00	0.00	0.00
Cyanobacteria (unk class)	0.00	0.00	0.00
Cyanobacteria (order) (unk family)	0.00	0.00	0.00
Cyanobacteria (family) (unk genus)	0.00	0.00	0.00
Cyanobacteria (class) (unk order)	0.00	0.00	0.00
Cupriavidus taiwanensis	0.00	0.00	0.00
Cupriavidus metallidurans	0.00	0.00	0.00
Cryomorphaceae (unk genus)	0.00	0.00	0.00
Cryocola sp	0.00	0.00	0.00
Cryocola (unk species)	0.00	0.00	0.00
Cryobacterium (unk species)	0.00	0.00	0.00
Crocinitomix sp	0.00	0.00	0.00
Crocinitomix (unk species)	0.00	0.00	0.00
Crocebacterium (unk species)	0.00	0.00	0.00
Crenothrix (unk species)	0.00	0.00	0.00
Coxiellaceae (unk genus)	0.00	0.00	0.00
Coscinodiscus radiatus(notFound)	0.00	0.00	0.00
Coscinodiscus radiatus	0.00	0.00	0.00
Coriobacteriales (unk family)	0.00	0.00	0.00
Clostridium bifermentans	0.00	0.00	0.00
Clostridiales incertae sedis (unk genus)	0.00	0.00	0.00
Clostridiales (unk family)	0.00	0.00	0.00

Citreicella (unk species)	0.00	0.00	0.00
Chryseobacterium hispanicum	0.00	0.00	0.00
Chroococcales uncultured	0.00	0.00	0.00
Chroococcales (unk family)	0.00	0.00	0.00
Chroococcales (genus) (unk species)	0.00	0.00	0.00
Chroococcales (family) (unk genus)	0.00	0.00	0.00
Chroococcaceae (unk genus)	0.00	0.00	0.00
Chlorophyta uncultured(notFound)	0.00	0.00	0.00
Chlorophyta uncultured	0.00	0.00	0.00
Chloroflexi (unk class)	0.00	0.00	0.00
Chloroflexi (class) (unk order)	0.00	0.00	0.00
Chloroflexales (unk family)	0.00	0.00	0.00
Chloroflexaceae (unk genus)	0.00	0.00	0.00
Chlorella ellipsoidea(notFound)	0.00	0.00	0.00
Chitinimonas (unk species)	0.00	0.00	0.00
Cellvibrio mixtus	0.00	0.00	0.00
Cellvibrio fulvus	0.00	0.00	0.00
Cellvibrio (unk species)	0.00	0.00	0.00
Cellulomonadaceae (unk genus)	0.00	0.00	0.00
Celerinatantimonadaceae (unk genus)	0.00	0.00	0.00
Caulobacterales (unk family)	0.00	0.00	0.00
Caulobacter (unk species)	0.00	0.00	0.00
Candidatus Rhodoluna (unk species)	0.00	0.00	0.00

Candidatus Planktoluna (unk species)	0.00	0.00	0.00
Candidatus Monilibacter (unk species)	0.00	0.00	0.00
Candidatus Aquiluna (unk species)	0.00	0.00	0.00
Campylobacteraceae (unk genus)	0.00	0.00	0.00
Caldilineales (unk family)	0.00	0.00	0.00
Caldilineae (unk order)	0.00	0.00	0.00
Caldilineaceae (unk genus)	0.00	0.00	0.00
Caldilinea sp	0.00	0.00	0.00
Caldilinea (unk species)	0.00	0.00	0.00
Byssovorax sp	0.00	0.00	0.00
Byssovorax (unk species)	0.00	0.00	0.00
Burkholderia glathei	0.00	0.00	0.00
Brucellaceae (unk genus)	0.00	0.00	0.00
Brucella (unk species)	0.00	0.00	0.00
Brevundimonas nasdae	0.00	0.00	0.00
Brevundimonas diminuta	0.00	0.00	0.00
Brevibacterium antiquum	0.00	0.00	0.00
Brevibacteriaceae (unk genus)	0.00	0.00	0.00
BRC1 (order) (unk family)	0.00	0.00	0.00
Brachymonas (unk species)	0.00	0.00	0.00
Bordetella avium	0.00	0.00	0.00
Beijerinckiaceae (unk genus)	0.00	0.00	0.00
Beijerinckia indica	0.00	0.00	0.00
Beijerinckia derxii	0.00	0.00	0.00
Bdellovibrionales (unk family)	0.00	0.00	0.00
Bartonella	0.00	0.00	0.00

henselae(notFound)			
Bacteroidetes (class) (unk order)	0.00	0.00	0.00
Bacteroides intestinalis	0.00	0.00	0.00
Bacteroides (unk species)	0.00	0.00	0.00
Bacteroidales (unk family)	0.00	0.00	0.00
Bacteriovoracaceae (unk genus)	0.00	0.00	0.00
Bacteria incertae sedis (unk class)	0.00	0.00	0.00
Bacillariophyta uncultured(notFound)	0.00	0.00	0.00
Bacillariophyta uncultured	0.00	0.00	0.00
Azospirillum oryzae	0.00	0.00	0.00
Azospirillum lipoferum	0.00	0.00	0.00
Azospirillum brasilense	0.00	0.00	0.00
Azoarcus evansii	0.00	0.00	0.00
Azoarcus (unk species)	0.00	0.00	0.00
Auxenochlorella protothecoides(notFound)	0.00	0.00	0.00
Auxenochlorella protothecoides	0.00	0.00	0.00
Arthrobacter (unk species)	0.00	0.00	0.00
Arcobacter cryaerophilus	0.00	0.00	0.00
Arcobacter butzleri	0.00	0.00	0.00
Arcobacter (unk species)	0.00	0.00	0.00
Aphanothece sp 0BB21S01	0.00	0.00	0.00
Aphanizomenon (unk species)	0.00	0.00	0.00
Anaeromyxobacter (unk species)	0.00	0.00	0.00
Anabaena solitaria BC Ana	0.00	0.00	0.00

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Anabaena (unk species)	0.00	0.00	0.00
Aminomonas (unk species)	0.00	0.00	0.00
Aminobacter (unk species)	0.00	0.00	0.00
Alistipes putredinis	0.00	0.00	0.00
Alicycliphilus denitrificans	0.00	0.00	0.00
Afipia broomeae	0.00	0.00	0.00
Afipia (unk species)	0.00	0.00	0.00
Aeromonas eucrenophila	0.00	0.00	0.00
Aeromonas allosaccharophila	0.00	0.00	0.00
Actinosynnemataceae (unk genus)	0.00	0.00	0.00
Actinoplanes teichomyceticus	0.00	0.00	0.00
Acidobacteriales (unk family)	0.00	0.00	0.00
Acidobacteriaceae (unk genus)	0.00	0.00	0.00
Acidobacteria (class) (unk order)	0.00	0.00	0.00
Acidisphaera rubrifaciens	0.00	0.00	0.00
Acidisphaera (unk species)	0.00	0.00	0.00
Achromobacter (unk species)	0.00	0.00	0.00
Acetobacteraceae (unk genus)	0.00	0.00	0.00
Acetobacter (unk species)	0.00	0.00	0.00

Species Percentages for Cultivated samples.